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TITLE OF THE INVENTION (280 characters max)

BIOELECTRICAL PARAMETERS RELATED TO GLUCOSE LEVEL MEASUREMENT PRINCIPLES AND DATA ANALYSIS

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M.Sc. Thesis no. 195

Bioelectrical Parameters related to Glucose Level
- *Measurement principles and Data analysis*

Bioelektriska parametrar relaterade till glukoskoncentration
- Mätprinciper och Databehandling

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Abstract

Bioelectrical parameters related to glucose level - measurement principles and data analysis

Ulrik Birgersson and Fredrik Neiderud

Substances that do not dissociate when dissolved in water will not contribute to the conductivity of the solution, since they are not ionised. One of these that are common in the human body is glucose, the main energy provider for the cells. However, simple laboratory experiments presented in this master-thesis indicate that glucose does have an influence on the impedance magnitude of a solution made of physiological saline and glucose, likely caused by changes in the mobility of ions.

Since this might not be generally applicable, electrical impedance measurements were conducted *in vivo* in different kinds of tissues with an impedance spectrometer, the SciBase II. No correlation between skin impedance and blood glucose concentration could be established, probably due to the dynamic changes of the skin surface.

As a result of the skin problematic a decision was made to measure impedance with intra-muscular electrodes using the four-point impedance spectrometer IBSA. The results from these measurements indicated that a correlation exists. The correlation was investigated further with the help of the SciBase II and subcutaneously placed electrodes, resulting in a strong indication that the correlation between electrical impedance in human tissues and blood glucose concentration truly exists.

All measurements were performed on a subject diagnosed with type 1 diabetes.

Key words: Electrical impedance, Diabetes, Blood glucose concentration, SMBG

Sammanfattning

Bioelektriska parametrar relaterade till glukosnivå - mätprinciper och dataanalys

Ulrik Birgersson och Fredrik Neiderud

Ämnen som inte joniseras då de löses upp i vatten kommer inte bidra till lösningens konduktivitet. Ett av dessa ämnen, som är vanligt i människokroppen är glukos, cellernas huvudsakliga energikälla. I detta examensarbete presenteras enkla laboratorieförsök som har indikerat att glukos har en påverkan på impedansmagnituden i en lösning bestående av fysiologisk koksaltlösning och glukos. Det beror sannolikt på förändringar i jonmobiliteten.

Eftersom det sambandet inte nödvändigtvis gäller generellt, utfördes mätningar av elektrisk impedans med en impedansspektrometer, SciBase II, *in vivo* i olika sorters vävnad. Ingen korrelation mellan hudimpedans och blodglukoskoncentration kunde påvisas, förmodligen på grund av dynamiska förändringar av hudytan.

Hudproblematiken resulterade i att fortsatta mätningar utfördes under hudytan. Impedans mättes intramuskulärt med IBSA, en impedansspektrometer som arbetar med fyrpunktsteknik. Resultaten från mätningarna indikerade existensen av en korrelation. Korrelationen undersöktes närmare med hjälp av elektroder som placerades subkutant och kopplades till SciBase II, vilket resulterade i en ännu tydligare indikation på att det verkligen existerar en korrelation mellan elektrisk impedans i mänsklig vävnad och blodglukosnivå.

Alla *in vivo* försök utfördes på en typ 1 diabetiker.

Sökbegrepp: Elektrisk impedans, Diabetes, Blodglukosnivå, SMBG

Contents

Chapter 1

Introduction

Diabetes is a rapidly growing health problem worldwide. From 2000 to 2030 an increase from 170 million to 370 million is expected, i.e. an increase with approximately 120% in just 30 years. The problem is likely even greater than that, since one must keep in mind that this is just the reported number of people diagnosed. In the US alone, the cost of diabetes care was estimated to a staggering US$ 44 billion in 1997.

The test-strips for self-monitoring of blood glucose constitutes the main part of the diabetes care costs. The costs for treating the complications that follow from long gone diabetes is also considerable. Therefore it would be of great economical as well as medical interest to develop a new device for SMBG that facilitates continuous monitoring of blood glucose level. The costs would be lowered and, most important, the quality of life for diabetes patients would be greatly improved. If such a device would be incorporated with an insulin pump, an artificial pancreas could be constructed.

Because of the great economical and medical benefits, a large number of researchers around the world have made attempts at developing new measurement techniques that are able to monitor blood glucose concentration continuously. Most of the research is aimed at developing non-invasive continuous measurement systems [17], since these would minimize the discomfort and risk for the patients.

The experiments presented in this master-thesis were based on previously found correlation between blood glucose level and electrical impedance measured non-invasively on the skin [33]. The main goal of our experiments was to establish where in the human body the correlation could be found.

Chapter 2

Medical Background

2.1 Glucose

Most of the carbohydrates in our diet are polysaccharides. Unfortunately the body cannot absorb them directly, instead they have to be split into monosaccharides, i.e. glucose, fructose or galactose. At least 80 % of the carbohydrates are become glucose in the digestion, the rest become fructose or galactose. These can be converted to glucose though, thus making it a very important source of energy to the human body.

Like most other substances in the body, the glucose absorbed in the small intestine is transported around the body with the blood. Therefore the amount of glucose in the blood is a pretty good indication of the total glucose concentration in the body.

Blood glucose concentration is usually measured in millimolar glucose, i.e. millimoles glucose per litres of blood (mM, European scale) or milligrams glucose per decilitres of blood (mg/dl, American scale). The European scale and the American scale are directly proportional and 1 mM equals approximately 18.02 mg/dl. This relationship is easily explained with some basic chemistry [10]. The chemical formulae for glucose is $C_6H_{12}O_6$, and hence the weight of one mole glucose is:

$$6 \cdot M_C + 12 \cdot M_H + 6 \cdot M_O \approx 6 \cdot 12.011\text{g} +$$

$$+12 \cdot 1.00794\text{g} + 6 \cdot 15.9994\text{g} \approx 180.2\text{g}$$

When this is known, the conversion is fairly simple:

$$1\ \text{M} = 1\ \frac{\text{mol}}{\text{litre}} \approx \frac{180.2}{10} \cdot \frac{\text{g}}{\text{dl}} = 18.02\ \text{g/dl}$$

$$1\text{mM} \approx 18.02\text{mg/dl}$$

The normal range of blood glucose in a healthy individual is 70 - 160 mg/dl.

Glucose is used for energy production by all cells, in a process called glycolysis. This is done inside the cells. However, the cell membrane is not permeable to glucose, except under some special circumstances and in some special cells (e.g. the cells of the CNS). The cells need help to be able to absorb glucose and this help is called insulin, a hormone produced in the pancreas.

Right after a meal the blood glucose value rises rapidly, as the digestion of carbohydrates begins. This triggers the secretion of insulin from the pancreas. When the insulin reaches a target cell, a cascade reaction starts with the result of transporting glucose molecules into the cell. The reaction is rather complex and will consequently not be explained here, but it is very well described in other literature (e.g. the source of this text, Textbook of Medical Physiology [1]). Once the glucose is inside the cell, the glycolysis can start.

Not all the glucose that enters the body during a meal is used directly. Some of it has to be stored otherwise we would have to eat all the time. Glucose is mainly stored in the liver cells, but also in the muscle cells to some extent. To keep the equilibrium between intra and extra cellular fluid intact, the glucose molecules are stored as glycogen, i.e. a large polymer of glucose. The process of combining glucose molecules to glycogen is called glycogenesis.

When the energy stored as glycogen is needed, the glycogen can be split into glucose again. This process is called glycogenolysis. Two hormones are especially important for the activation of the glycogenolysis, namely glucagon and epinephrine. Glucagon is (just like insulin) a hormone produced in the pancreas. It is secreted when the blood glucose value falls too low, for example in between meals. Epinephrine on the other hand is secreted from the adrenal glands when the sympathetic nervous system is activated, something that happens when the body prepares for hard work.

To summarize, the glycolysis is the production of energy from glucose molecules, while the glycogenesis and the glycogenolysis are (in that order) the formation and breakdown of glycogen.

2.2 Diabetes Mellitus

2.2.1 Historical perspective

Diabetes Mellitus is not a newly discovered disease. The first written documentation of the illness as "over-abundant urine" and treatment in form of diet was found in the Egyptian Ebers papyrus and has been dated back to around 1552 B.C.

The greek doctor Aretaious from Capadokia is the father of the word Diabetes which proclaims that the diseased drinks large amounts of water and has excessive urine excretion (polyuria). Mellitus is a synonym for honey sweat, related to the smell and taste of the urine of the patients suffering of diabetes mellitus. Thomas Willis described this honey sweat flavor in his work Pharmaceutice rationalis in the year 1674, where he distinguished between diabetes mellitus and diabetes insipidus.

In the case of diabetes insipidus no essence of sweetness exists in the urine due

to the fact that this disease is caused by unsatisfactory production of antidiuretic hormone (ADH) in the hypothalamus. The low production of ADH may have many different origins, for instance a tumor. About hundred years later, Matthew Dobson boiled urine from a diabetes mellitus patient and when the water had evaporated small crystals tasting and resembling glucose were revealed. John Rollo experimented with a range of diets and he found it to be a gastro-intestine related sickness.

Already in the year 1875 Bouchard could show that there exist two different types of diabetes mellitus. One struck against the obese and old whilst the other form showed itself in an opposite patient group of young and meager people. This gave rise to the definition of juvenile- and adult-onset diabetes mellitus, which have been exposed to be wrongly defined or less accurate, because of the possible onset of both types of diabetes at any age.

A few years later Minkowski and Von Merring could induce diabetes mellitus symptoms by removing the pancreas in dogs and thereby the source had finally been located. In the year 1921 Banting and Best finally isolated the "anti-diabetic factor" known to us as insulin and showed its blood sugar lowering effect on diabetic dogs.

In 1922 one of Bantings associates, Dr Collip, gave one of his extracts to the then 14 year old boy Leonard Thompson. The experiment was declared a success the month after. It did not take long for the insulin to get mass-produced and although the diabetes still could not be cured, it could now be controlled.

One year later Banting and Macleod were awarded the Nobel Prize. Since two of their associates where not given any of the official recognition, Banting shared his award with Best and Macleod with Collip.

After this deed there have been a lot of improvements in the field of diabetes mellitus research, but none as grand as the isolation of insulin. Technology has given diabetes patients the means to self-treat and self-monitor diabetes, but the cure still does not exist nor does one really know the cause of the disease. However the future holds many promises for diabetes patients [3, 4, 31].

2.2.2 Pathophysiology

Rightly, Bouchard made a distinction between the two types of diabetes. Although the distinction made was not fully correct, it was a start. Today we know that "juvenile" diabetes is caused by a low production or lack of insulin secretion brought on by the destruction of the beta cells of the pancreas. Although the true cause of this is still unknown, there are strong indications that the destruction could be caused by an autoimmune reaction, since some specific antibodies have been detected in a larger part of the patients at the onset of the disease [3]. The chronic disease can occur in any age group even if it has a higher probability to appear early in life. This kind of diabetes mellitus is classified as type 1 whilst the diabetes mellitus often occurring in the older population is called type 2. This kind of diabetes is caused by a slowed insulin secretion although the pancreas is not showing any signs of degeneration, as is the case in type 1 diabetes. Patients with type 2 diabetes also have a decreased sensitivity towards insulin and therefore need a

higher dosage of insulin to receive the same response. Reduced insulin sensitivity is directly connected to genetic factors as well as obesity. Many diabetics are suffering from obesity which increases the peripheral insulin resistance.

2.2.3 Onset of Diabetes Mellitus

Type 1 onset occurs sudden and glucose levels quickly rise to a state where they are life threatening if untreated. The onset of diabetes mellitus type 2 occurs slowly due to the fact that an insulin production still exists. The body adjusts to the slow increase of blood sugar and there are no real externally detectable signs except the patients often get some indications that a disorder could exist by increased urine discharge and tiredness. Since the disease is a slow process for type 2 diabetics, many long-term effects take their beginning here, as hyperglycemia is highly connected to diseases such as retinopathy and arteriosclerosis.

2.2.4 Pharmaceuticals

There exists a great variety of different blood glucose decreasing pharmaceuticals on the market today. The most well known is insulin. Since patients may benefit from different usage of insulin, a variety of insulin can be found. Generally insulin is divided into three categories:

- Prompt-acting Insulin: An effect can generally be observed after 10-15 min after injection with a maximum effect after approximately 45 minutes. Hence, this is used to suppress the rapid increase of blood glucose after the consumption of a meal.
- Intermediate-acting Insulin: This is generally a combined insulin kind, yielding an effect after roughly 1-3 hours with a peak at approximately 4-10 hours and cede to work after 16-24 hours. Different mixtures can give rise to changes in when the effect set in and how long term effects vary.
- Long-acting Insulin: These have come out on the market in the last years showing very interesting results. As it seems, they will give rise to a close to constant effect for 24 hours, which is exactly what is sought after to maintain a good basal insulin level [3]

Today there also exists other pharmaceuticals than insulin on the market, that have a blood glucose lowering effect. These drugs increase the endogen release of insulin or the tissue sensitivity to insulin. Some medicines can also block the absorption of glucose in the intestines and hence lower the blood glucose values.

Insulin injection sites

Insulin injections are made at different sites on the body to accommodate the wanted effect from the given insulin. The insulin injected should end up in the

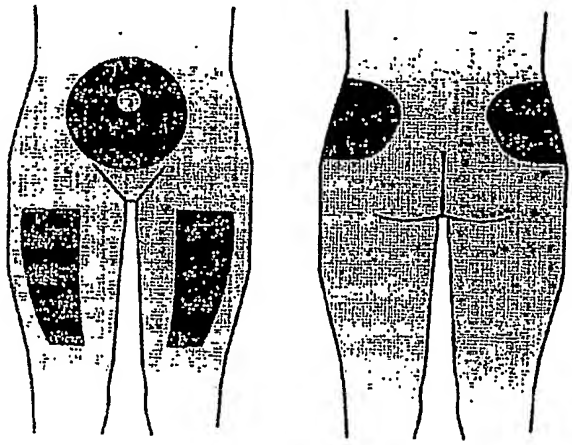

Figure 2.1: Recommended injection sites for subcutaneous insulin administration. *From:* Agardh C-D., Berne C., Östman J., *Diabetes, 2nd edition, Liber AB 2002.*

subcutaneous fat tissue, the area between the skin and the muscle tissue. This will yield a more evenly paced uptake of the insulin in the body and unnecessary onsets of hypoglycemia may be avoided. When administering fast-acting insulin, the abdominal fat around the umbilicus is used (fig 2.1). One should always take a grip around a skin crevice before penetrating the location with the syringe and hold on to it during the whole injection phase. Insulin may also be administered in the thighs as well as in the upper part of the buttocks. Long-term-acting as well as middle-acting insulin injections should be injected in the upper part of buttocks. This upper fraction of the buttocks consists of mostly fat and therefore one can easily inject the insulin here and have a beneficial pace of uptake.

2.2.5 Treatment

The treatment of diabetes mellitus aims at compensating the lack of insulin production in various ways. As we have seen, there are several kinds of insulin and other pharmaceuticals on the market, giving rise to a variety of treatment methods. The treatment of diabetes mellitus varies in many ways where the greatest deviation in treatment can be seen between the two types of diabetes mellitus.

Type 1 patients lack their own insulin production and they are therefore in constant need of insulin, whilst type 2 patients still have a remaining insulin production although unsatisfactory. Generally type 2 diabetes is treated with insulin sensitivity increasing drugs or pharmaceuticals mentioned above. Diet also plays a very important role in controlling the blood sugar levels in both type 1 and type 2 diabetes, although if the blood sugar levels still remain hard to control, type 2 patients are also treated with insulin injections.

In view of the fact that type 1 diabetics have a small to non-existing insulin

production, insulin injections play a vital role in their treatment. The practice of how many injections patients should take varies on different medical practice, although studies [35] have shown that multiple injections (in the range of 4-6 a day) give an improved HbA1c (gives the average of the blood glucose level in a patient over a period of approximately 120 days). The practice in Sweden is to give 3 fast-acting insulin injections with the main meals of the day and since a basal insulin level is needed, one also generally uses 2 middle-acting insulin injections 12 hours apart or 1 of the new long-term- acting insulin every 24 hours. The fact that the modern insulin has a more rapid uptake and effect makes it possible to eat anytime during the day as long as one gives the body the needed insulin to maintain good blood glucose levels.

The dosages vary greatly among patients and insulin levels need to be adjusted individually. Therefore, this is the only medicine in FASS that has no exact given dosage.

There is a common belief among people today that type 1 diabetics need to stay on a very strict diet, but with great improvements made to the different insulin types this is generally not the case. This belief most likely stems from when there were only long-term working insulin types so that a strict diet had to be maintained in order to regulate the blood sugar. For patients to maintain a good blood glucose level there are two approaches one can use. One is to calculate each calorie and uphold a strict insulin dosage and the other is to regulate the dosage to the amount of food that is consumed. The latter gives the individual more freedom in everyday life not having to worry about each calorie that is consumed.

2.2.6 Self-monitoring of blood glucose (SMBG)

The total number of people worldwide known to be suffering from diabetes mellitus in the year 2000 was a staggering 177 million and estimates gather that the number will grow steadily and in the year of 2030 more than 370 million [32]. This fact clearly shows that treatment of these patients needs to be done in an effective way to minimize costs for the society.

Patients of today are taught to monitor their own blood sugar levels in various extents, depending on the type of diabetes. Generally a person suffering from diabetes mellitus should use an invasive blood glucose meter four times a day. Given that the greatest variation of the blood sugar will appear after meals, the blood sugar should be tested before the three meals of the day, so that an appropriate dosage of insulin can be calculated and injected. Patients should also monitor their blood sugar before going to bed for the day to make sure that neither hypoglycemia (low blood glucose values) nor hyperglycemia (high blood glucose values) set in during the night. How does a patient know how high an insulin dosage he or she needs? When the patient is diagnosed, a doctor set a base dosage with which the patient can start. The patient also makes a visit to a nutritionist to discuss different kinds of foods and how they will influence the blood sugar. Since the patients cannot be admitted for the rest of their lives they need to learn how insulin counteracts rising blood sugar levels. They also need to learn that if it is taken in too large dozes

it causes hypoglycemia and this may even end up in an unconscious state, referred to as insulin coma, therefore delicate balancing is needed. Extreme hypoglycemia is lethal. That is why blood sugar levels should be checked as often as four times a day to accommodate a proper treatment, for pregnant women even more often. To provide a sense of security, patients are regularly checked in hospital and they may contact medical personnel at almost any time during the day to learn how to improve their blood glucose values.

2.2.7 Blood sugar levels

Hypoglycemia

The prefix hypo stands for low and this state sets in when the blood glucose levels fall beneath 3.5 mM (European scale) or 70 mg/dL (American scale). In a state of hypoglycemia, the body tries to increase the blood glucose levels. The normal response to this state is an increase in glucose production in addition to decreasing periphery glucose usage. To augment the increased glucose production hormones are released. The most important are glucagon, which makes the liver release some of its stored glucose and epinephrine to speed up circulation (see chapter 2.1). Hence these two hormones give a quick response to a state of hypoglycemia. Growth hormones and cortisone are also released yielding increased blood glucose levels after a couple of hours. In a healthy person the secretion of insulin would also be lowered. Since a patient suffering from diabetes type 1 injects insulin, there is no possibility to lower the effect of it and therefore a diabetic patient should always bring sugar pills in case an extra increase in blood sugar is needed. The time from when a person was diagnosed with diabetes plays a vital role in the hormone secretion, in view of the fact that glucagon and epinephrine secretion become less for each year and a substantial decrease can already be observed after five years with the disease. This is a major concern in that the patient will not get the proper response to low blood glucose levels. As plenty of hormones are involved in increasing the blood sugar, one can sense this state in many ways. Most common signs described by diabetic patients are: sweating, headache, nausea, hunger, heart pounding, lowered concentration ability, irritation on the verge to aggression, drowsiness and many more. These signs fade with lowered secretion of epinephrine and glucagon. Causes for the onset of hypoglycemia are fairly simple. The patient has injected to high a dosage of insulin or not eaten enough to match the dosage given. Putting off a meal, drinking alcohol or physical activity may also result in low blood sugar levels

Euglycaemia

A person not suffering from diabetes has a normal blood sugar level in the interval 4.4-6.7 mM or 80-120 mg/dL, independent of when when measured. A diabetic subject's blood sugar level should lie in the interval of 4.4-7.8 mM or 80-140 mg/dL right before a meal [29].

Hyperglycemia

The prefix hyper stands for high and this state sets in when the blood glucose levels rise above 10 mM or 180 mg/dL. The response to this state heavily depends on how long a person remains in it. When patients are diagnosed with diabetes mellitus they have been in this state for a long time and substantial effects can be seen on the body. This state is harder to describe in a short summary and the reader is therefore referred to more thorough information on the subject [1, 3]. Some signs that can be observed in a person with hyperglycemia are: increased thirst, increased urine amounts, tiredness, loss of appetite, nausea, vomiting, stomach pain, short of breath and if the person has had hyperglycemia for a very long time, acetone can be smelt from their breath. If the person is not a diagnosed diabetic and these signs can be seen or smelt, make sure to seek medical help directly! A diabetic can detect these states through SMBG and hence give more insulin to decrease the blood glucose. Causes for this state can be much more subtle than those mentioned for low blood glucose levels. Infections and fever will increase the bodily need of insulin and then dosages need to be raised. How much varies strongly depending on how severe an infection or how sick the patient is. Of course, there may also be simple explanations for a high blood sugar, one being that too much food has been consumed or the wrong kind of food.

2.2.8 General advice

There is a general misconception in how one should handle a situation where one is confronted with an almost unconscious or unconscious diabetic. Should you administer insulin or should you give a something to increase the subject's blood glucose? First of all, if a person is unconscious and does not respond, do not give the subject anything. Pressing something into the mouth of an unconscious person may block their airways and hence may be fatal. Injecting insulin in such a person will in most cases be lethal, since unconsciousness that occurs in diabetic persons almost always is caused by low blood sugar levels. Administered insulin will yield even lower blood sugar levels. A close to unconscious person might benefit from something that raises their blood sugar levels, but be very careful not to obstruct the person's airways. The best alternative is to get the patient to hospital and into the hands of medical personnel.

2.3 The Skin

Most of the measurements that are about to be presented were performed in the skin. To understand these measurements, it is good to have some knowledge about the structure of the skin and what purpose it fulfils in the human body.

The first question to be asked is certainly; why measure in the skin at all? That can easily be explained by an old saying: "The skin is the mirror of the soul", meaning that whatever is wrong with a person, it will be reflected by his (or her) skin. A more straightforward explanation is that the skin not only is the

biggest organ in the body, but it is also one of the few that are accessible for non-invasive measurements. By measuring on the skin surface, the measurements can be performed completely painless.

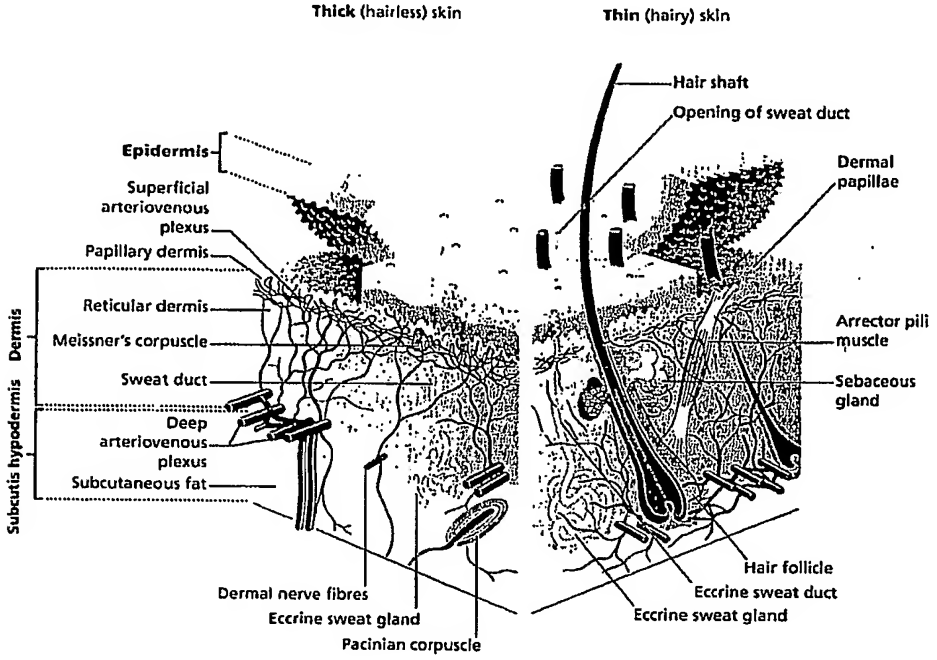

Figure 2.2: Cross section of the whole skin. *From:* Hunter J.A.A., Savin J.A., Dahl M.V., *Clinical Dermatology, 3rd edition, Blackwell Science 2002.*

The skin has three main layers (fig 2.2). From outside and inward they are the epidermis, the dermis and the subcutis. All three layers have different functions and consequently different structures. Since the measurements were done on the skin surface, the epidermis is the most interesting layer for this project.

2.3.1 Epidermis

As this is the outermost layer of the skin, it is the barrier between the human body and a dangerous world. It protects us from many harmful things, such as chemicals, microbes and radiation. The epidermis also prevents dehydration by reducing the water loss from the body.

Just as the whole skin, the epidermis can be divided into several different layers with different functions and structures (as seen in fig 2.3). The epidermis begins with a basal membrane, separating it from the dermis. On top of the basal membrane rests the first layer of living cells, called the basal layer (stratum basale). This can be seen as the factory for skin cells, since this is where the cell division occurs.

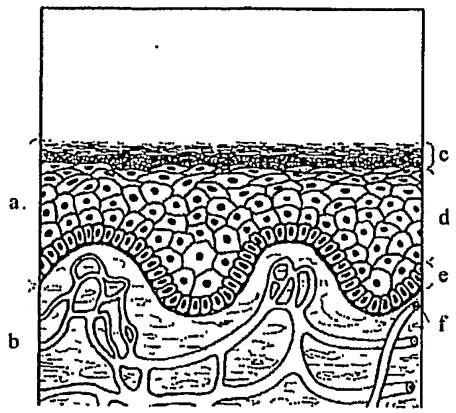

Figure 2.3: Cross section of the two uppermost layers of the skin: a) Epidermis, b) Dermis, c) Horny layer, d) Living epidermis, e) Basal layer, f) Basal membrane. *From:* Nicander I., *Electrical impedance related to experimentally induced changes of human skin and oral mucosa, PhD Thesis, Karolinska Institutet 1998*

The cells produced in the basal layer are pushed outward by new cells being produced. As they move towards the skin surface, the cells change their appearance in several steps, for instance they become flattened and dehydrated. All the layers on the way to the surface have different names, but together they are called the living epidermis. When the cells finally reach the surface, they are dead. But even though they are dead, it is now that they perform their most important task, the task of being a barrier towards the outside world.

This layer of dead skin cells is called the horny layer (stratum corneum). It resembles a brick wall, where the bricks are the dead cells and the mortar is intracellular lipids. The brick wall is not more than 15 micrometers thick in general, but on the palms and soles it can reach almost 2 mm. The complete epidermis varies between 0,1 mm and 2 mm and the variation depends almost completely on thickness of the horny layer [2].

2.3.2 Dermis

Beneath the epidermis lies a considerably thicker layer, called dermis. It consists mainly of collagen fibres that build up the mechanical strength of the skin, but it also contains blood vessels, nerves and hair follicles among other things. This layer has numerous functions, but one of the most important is the fact that it supports the epidermis structurally and nutritionally. The epidermis does not contain any blood vessels, but since it has living cells it still needs nutrition and oxygen. This is taken care of by the capillaries of the dermis.

The interconnection between dermis and epidermis has a papillary structure,

not flat as one might think. This geometry increases the interconnection area between the layers, making the exchange of substances easier. In this case it also increases the mechanical strength of the skin, since the different skin layers will be held together more tightly.

2.3.3 Subcutis

The subcutis (hypodermis) has two important functions, namely thermal insulation and protection against mechanical trauma. It consists mainly of cells that have specialized on fat storage, so called lipocytes. This is known as the subcutaneous fat. The thickness of this layer will vary a lot between individuals, mostly depending on heredity, nutritional status and hormonal balance.

Chapter 3

Technical Background

3.1 Bioimpedance

The goal of this chapter is to give the reader a basic understanding of electrical impedance, not to give a complete lecture on bioelectricity. For a more thorough lecture, the interested reader is referred either to *Bioimpedance & Bioelectricity Basics* [5] or *Medical Physics and Biomedical Engineering* [6].

Electrical resistance is a material property, which describes a materials capability of conducting electrical currents. A material with high resistance is a poor conductor, and inversely, a material with low resistance is a good conductor. It is defined as the fraction between applied voltage over the material and the current flowing through the material ($R = U/I$), an equation also known as Ohm's law.

Besides different kinds of conductors, there is another group of materials called insulators. They do not work in the same way, since their charges are bound and not able to move around freely in the material as the charges in a conductor do. Bound charges do not create a current, since current is a flow of charges. However, the insulators have dielectric properties that are a result of very small displacements of the bound charges. These movements make it possible to conduct an alternating current through the material, without any net transport of charged material.

These two aspects of charge transportation can be described by one compound property called *impedance*. It is represented by a complex number, having a real part representing the resistive aspect and an imaginary part representing the capacitive aspect. As with all complex numbers, it can also be presented as a magnitude and a phase angle instead of real and imaginary parts and most often this kind of presentation is used. Impedance is calculated using a more general form of Ohm's law: $\vec{Z} = \vec{U}/\vec{I}$, but one must remember to use vectors (having both a magnitude and a phase angle) for the voltage, current and impedance for this equation to work generally.

When considering AC, voltage is usually described as $u = \hat{u}\cos\omega t$ and current as $i = \hat{i}\cos\omega t + \varphi$, where $\hat{u}$ and $\hat{i}$ are the peak values and the cosine part describes

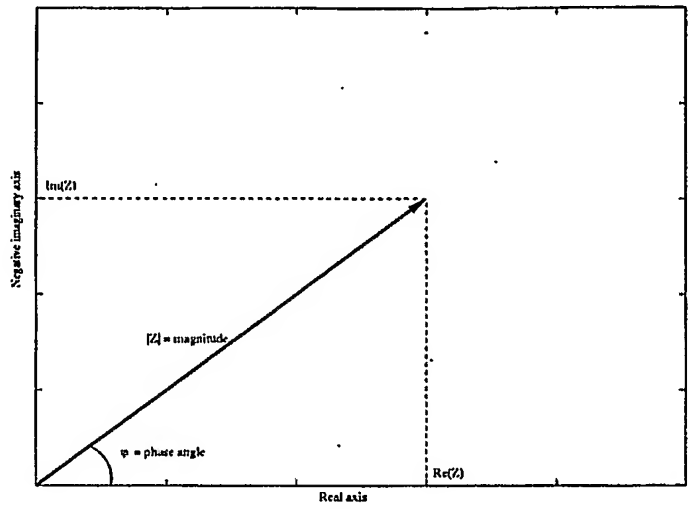

Figure 3.1: Impedance plotted in a complex space.

the periodical changes. φ is the phase angle between the voltage and current and it describes the lag between those two, a lag that depends on the capacitive property of the material. Since it is a relative expression, it can be placed on either the voltage or current expression as long as one remembers to change the sign. A pure conductor has no capacitive element and hence no lag between the voltage and current, therefore $Z = \frac{u}{i} = \frac{\hat{u}\cos\omega t}{\hat{i}\cos\omega t} = \frac{\hat{u}}{\hat{i}} = R$.

For tissue it gets a little more complicated, since it is a combined material that consists of both insulating and conducting materials. If tissue is described as a conductor, then one term has to be added to account for the small displacement movements of the bound charges. If it is described as an insulator instead, another term has to be added to account for the movement of free charges. Of course, both ways of thinking will yield the same result, but they can be more or less difficult to use, depending on the circumstances. The impedance of tissue has been given its own name, *Bioimpedance*, because of these special properties.

Tissue can be modelled with different complexity using electrical components. A very simple model of tissue can be constructed using a couple of capacitors and a resistor connected as in fig 3.2. This is called a Debye model.

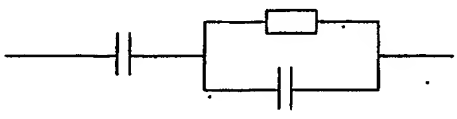

Figure 3.2: Electrical circuit equivalent for the Debye model.

As seen in fig 3.3(left), the impedance magnitude decreases with increasing

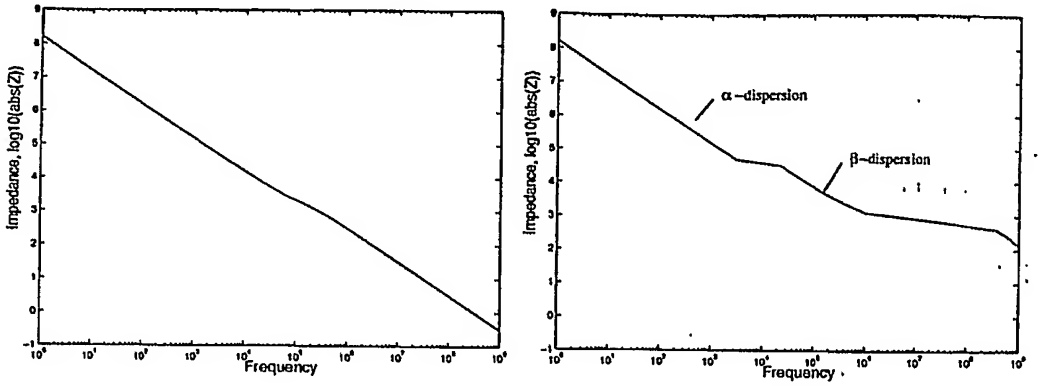

Figure 3.3: Impedance vs. frequency, for the Debye model (left) and for real tissue (right).

frequency. For the small circuit used in this example it can easily be explained using mathematics, but since it is supposed to be a model of tissue, the explanation must be based on some kind of biological phenomenon. The realistic explanation is that low-frequency currents cannot penetrate the cellular membrane, only travel in the extra cellular space, whereas currents of higher frequency can penetrate the cells and thereby use a more direct path between the electrodes.

However, if one measures impedance in a piece of tissue and plots it against frequency as above, it will not give a linear plot as in fig 3.3(left). The real plot will look something like fig 3.3(right). The separate slope sections are called dispersions and depend on different biological phenomena in tissue.

The first to publish an explanation of these dispersions was H.P. Schwan, as early as 1957 [22]. Since then, a lot of research has been conducted to examine them further, for numerous different applications. Most of the work is based on the research of Schwan and his article from 1957 is one of the most cited in the field of biomedical engineering [5]. In 1989 Foster and Schwan published an extensive review of the research performed in this field since 1957, a good source for information on electrical properties of tissue [23].

Since the total impedance of tissue depends on both the cellular structure and the composition of both extra and intra cellular fluid (ECF and ICF), it can be a good diagnostic tool in health care. In this project an attempt is made to link bioimpedance with blood glucose level, but it is already being used in a number of other applications, for instance estimation of skin irritation from different chemicals [14]. There is also ongoing research to develop bioimpedance as a tool for skin cancer detection [19, 20].

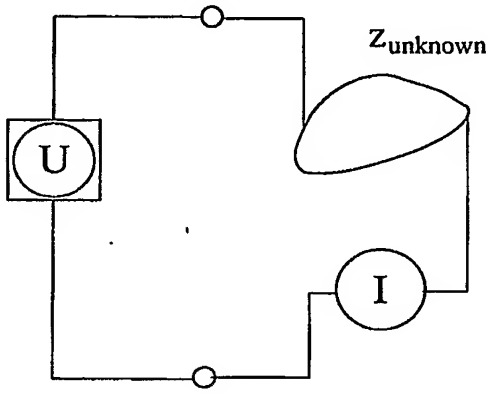

Figure 3.4: Basic impedance measurement setup. A fixed voltage source with known voltage is connected to the measurement object. An amperemeter is also connected in series with the measurement object to measure the current flowing through it. Impedance is then calculated according to the definition, $\vec{Z} = \vec{U}/\vec{I}$. Using a fixed current source and detecting the corresponding vlotage instead, would yield the same result.

3.2 Basic measuring principles

As the goal of this project is to find a correlation between bioimpedance and blood glucose level, these are the two properties that has to be measured. The blood glucose level will be measured using an invasive blood glucose meter (Glucometer Elite XL 3901E, Bayer). This chapter will describe how to measure impedance.

There are a number of different practical approaches of finding out the impedance, but basically they all work in the same way. In the previous chapter, the definition of impedance was given as $\vec{Z} = \vec{U}/\vec{I}$. From this relation it is quite easy to calculate the impedance if both the voltage and the current are known. A known voltage is applied to the object of interest and the current flowing through the material is measured. Then the impedance is calculated according to the definition.

The most straightforward approach would be to attach two electrodes to an object and applying the fixed voltage using these electrodes. An ampere meter should also be connected in series with the object to measure the current flowing through it (fig 3.4). This is called a two-point impedance measurement.

However, to increase the accuracy of the measurements, a number of better (and more advanced) methods have been invented:

- **Four-point-technique:** One way of increasing the accuracy is to use two electrodes for current injection and two other electrodes for voltage detection (a total of four electrodes, hence the name). This method is more accurate, especially for low impedance values, since the effects of electrode impedance at the injecting electrodes will not affect the sensing electrodes.

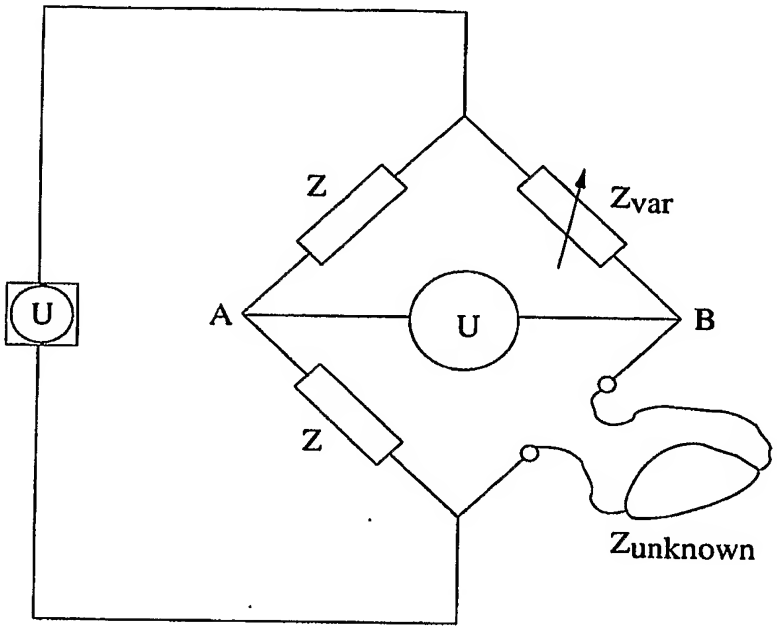

Figure 3.5: An impedance bridge for accurate measurements. When the bridge is balanced, the value of Z_{var} is the same as the impedance value of the measurement object.

- **Impedance bridge:** Another way of increasing the accuracy is to eliminate the possible errors in the instruments. If a connection as the one in fig 3.5 is used, the instrument only has to determine if there is any vectorial potential difference between points A and B (i.e. U_A and U_B equal in both magnitude and phase) and thereby the relative error is eliminated.

  The method is based on a balanced impedance bridge. As can be seen in the figure, the impedances in the left branch have equal value and the upper right is an adjustable impedance. The lower right impedance has been replaced with the object of interest. In the middle, a vector voltmeter is connected. To find out the impedance of the measurement object, Z_{var} is adjusted until the bridge is balanced and the voltmeter reads 0 V. Using simple calculations, one can now see that the impedance of the measurement object is equal to the adjusted value of Z_{var}.

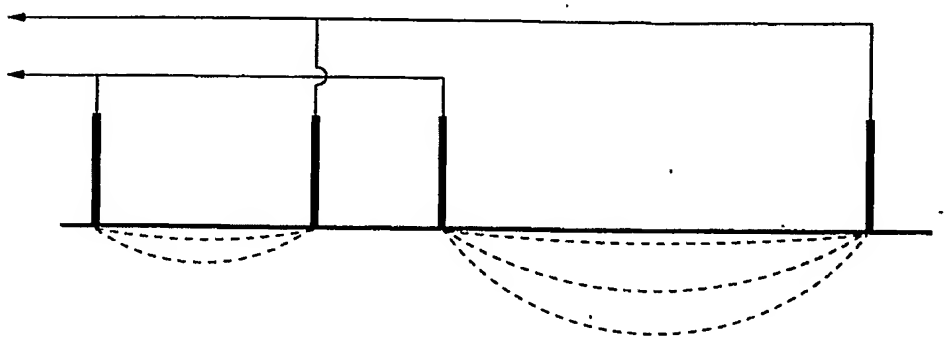

Figure 3.6: The distance between two surface electrodes determines the penetration depth. If the distance is increased, the penetration depth increases.

3.3 SciBase II

The basic methods of measuring impedance have been described above. However, in this project a more advanced system is used, an instrument called the SciBase II [28]. It is an impedance spectrometer equipped with a depth selective probe for non-invasive measurements on human skin. Another probe, adjusted for measurements on oral mucosa, is also available, but will not be used in this project.

Besides the instrument and the probe, a PC equipped with the software ImpSoft is also needed. The program is used for presenting and storing the measured data. The instrument is connected to the PC using an infra red link and it is powered by a battery. This way, the instrument is completely shielded from all harmful voltages. Even if something breaks down, there is no risk of causing any harm to the patient. The instrument is CE marked as a medical device of class IIa.

The instrument is an impedance spectrometer, which means that it measures the impedance at several different frequencies. This instrument is programmed to measure at 31 logarithmically distributed frequencies between 1 kHz and 1 MHz. The goal is to find out how the impedance changes at different frequencies. This will generally reveal more information about an object than just measuring at one frequency, but it is especially interesting for human tissue, because of the special properties of bioimpedance (see chapter 3.1). The real and imaginary parts of the impedance are measured separately, but in the software the measurement data is presented using the magnitude and phase instead. As described above, it is a fairly simple conversion and this representation is used more often.

The ability to measure at different depths is based on the fairly simple fact that the distance between two surface electrodes determines the penetration depth of the electricity. A graphical explanation is available in fig 3.6.

As can be seen in fig 3.7, the probe surface consists of four concentric electrodes. The two outermost are used for electricity injection and the middle electrode is used for sensing. By injecting the electricity with two electrodes, one virtually moveable electrode is created. The virtual electrode is positioned somewhere in between the

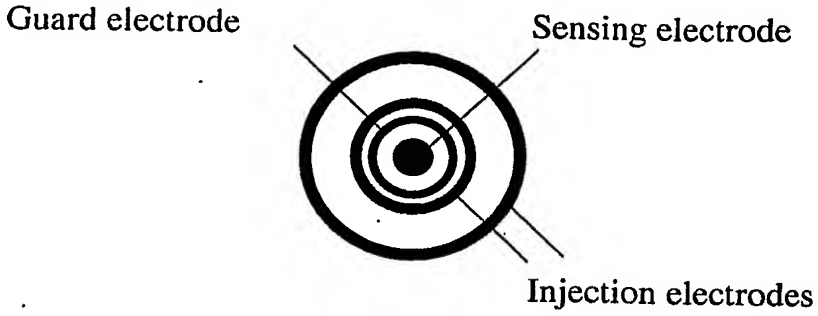

Figure 3.7: A schematic view of the surface of the probe.

two physical electrodes. If the majority of the electricity is injected through the outermost electrode, the virtual electrode is moved outwards and vice versa. With this virtually moving electrode, the depth selectivity of the probe is created as described above. The system is configured to measure at five predetermined depths between 0.1 and 2 mm, but theoretically any number of depth could be used. The last electrode is a guard electrode used to shield the sensing electrode from leakage currents along the skin surface. Since such currents do not pass through any tissue, they do not carry any interesting information. Instead they could possibly ruin the measurements.

Chapter 4

Data Analysis

4.1 Introduction

When we interact with the world around us, we are not limited to just one sense, but make use of five. Our bodies are thus collecting and analysing huge amounts of data at any given time. In analogy, the measurement techniques of today allow us to sample many parameters at once. Analysing the plenitude of information calls for advanced techniques and it is here that the multivariate analysis enters. In contrast to classical statistics, this method allows for processing of not one or a few variables, but many. From these, models that capture the studied phenomenon can be derived, without sacrificing any of the essential physics.

4.2 Classical statistics

Before turning our attention towards the workings of multivariate analysis, we will consider a few concepts related to classical statistics, as these will become important for the preprocessing and verification later on. These are:

- Arithmetic Mean, a coefficient representing the average over a set of values;

$$\overline{x} = \frac{\sum_{i=1}^{n} x_i}{n} \tag{4.1}$$

- Variance, a measure of the spread around the mean value;

$$var(x) = \frac{\sum_{i=1}^{n} (x_i - \overline{x})^2}{n-1} = \sigma_x^2 \tag{4.2}$$

- Standard deviation is the square root out of the variance yielding the same unit as the variable;

$$std(x) = \sqrt{var(x)} = \sigma_x \tag{4.3}$$

- Co-variation between to variables is a measure in linear comparability;

$$cov(x,y) = \frac{\sum_{i=1}^{n}(x_i - \overline{x})(y_i - \overline{y})}{n-1} \tag{4.4}$$

- Correlation is a unit less scaled co-variance;

$$correlation = r = \frac{cov(x,y)}{std(x)std(y)} \tag{4.5}$$

The correlation coefficient r gives rise to an independent measure of covariance. It can be shown that that the coefficient always remains between -1 and 1, where -1 is equivalent to a completely linear negative relationship between two variables and 1 equivalent to a linear positive relationship. Independent variables would yield a correlation coefficient of 0. In statistics r^2 is the most widely spread form of expressing correlation and thus will be used henceforth [9].

4.3 Data Presentation

To get a first overview of the measured data, we start by looking at different ways to obtain the sample variance and the skew distribution as well as identify possible outliers. Outliers comprise points that strongly deviate from the rest of the measurements and most often they are erroneuos. Hence these points should be examined further before including them in the raw data.

A first look at raw data plots might reveal the sample variance. The skew distribution of a sample variable can be examined with a histogram, which provides valuable information as to how and if the data requires preprocessing.

A powerful graphical tool is the box plot. The box plot visualizes the distribution of one or many samples or variables in the form of a box or boxes with upper and lower "whiskers" (see fig 4.1).

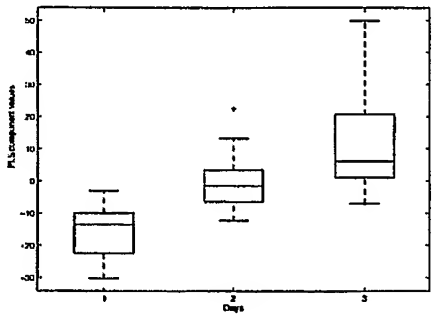

Figure 4.1: A typical box plot constructed using Matlab.

The box itself represents the mid 50% of the data. The line located inside the box represents the midpoint of a set of samples, called the median. A non-centered

median constitutes a first indication that the variable or observation may contain a skew distribution. Turning our attention to the "whiskers" situated above and below the box, we find them to represent at most 1,5 times the distance spanned by the box itself. If no outliers are present in the data, these lines will represent the maximum or minimum sample. All values lying outside the "whiskers" are considered outliers and are given a plus sign or a minus sign depending on where they are located with respect to the box [30].

4.4 Preprocessing

Preprocessing of the raw data is essential before the data is fitted to a model. This could be crucial between getting a good model or none at all. The aim of preprocessing is to reduce variation that possibly overshadows the information that one wants to obtain. One of the most common preprocessing steps is to mean centre the data and thereafter scale the variables by dividing them with their standard deviation resulting in an equal opportunity for all the variables to influence the model (fig 4.2).

It is not always beneficial to centre or scale the variables, as there exists applications where such an approach is only damaging and thus gives a poor model when a more accurate one could be achieved.

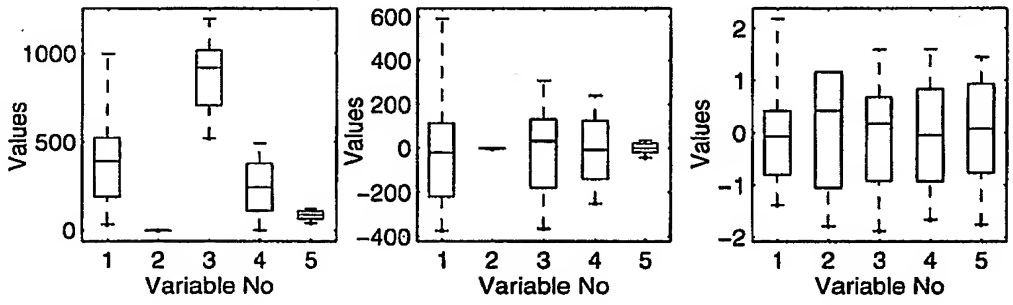

Figure 4.2: Left: Box plot of five variables. Center: Box plot after centering. Right: Box plot after centering scaling with standard deviation.

4.5 Principal Component Analysis (PCA)

Data gathered in an experiment with a large number of variables can be accumulated in a data matrix $\mathbf{X}$ of size $n \times k$, where the rows n represent the different measurements and the columns k signify the variables. From a statistical point-of-view PCA deals with finding subspaces of the data matrix $\mathbf{X}$ in which as much information as possible is retained. By reducing the number of dimensions, PCA

helps in finding "hidden patterns" or "hidden phenomena" which are not easily detectable when dealing with the raw data. A powerful tool when trying to categorize data or find resembling features between samples or observations [8].

A correlation coefficient r of 1 implies that two variables, spanning a 2-dimensional space, can be represented by a line without loss of any information. PCA locates new orthogonal base vectors, principal components in the Cartesian space spanned by the data matrix $\mathbf{X}$, who are positioned in the direction of the greatest variance. Applying PCA on a data matrix gives a reduced data set with a more compressed content (see fig 4.3).

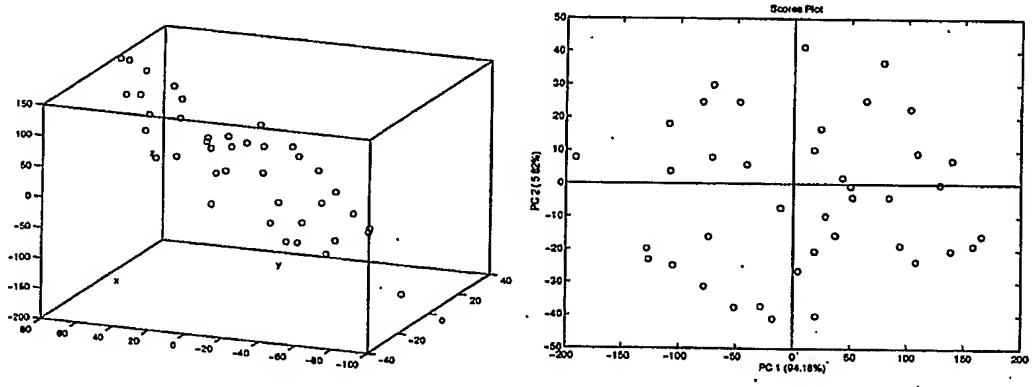

Figure 4.3: Raw data distribution before applying PCA (left) and after PCA (right).

Each component consists of a set of scores (t_i) and a set of loadings (p_i). The loadings describe how the scaled variables relate to the new principal components and as such can reveal how variables are correlated to each other as well as showing the individual variables significance for the given model. The scores on the other hand correspond to the new positions of the observations in the principal component space. Visualizing the observations with different principal components may show possible trends in the score plot, such as clusters and outliers. If there are outliers in the data these will distort the model. The score plot will help in deciding which observations deviate substantially from the others and should be excluded. Removing the outliers and recalculating the model will produce a less distorted model. In general the first as well as the second principal component can be grasped. Combining both the score plot and the loading plot facilitates an understanding of why some samples deviate and why some group.

The PCA algorithm NIPALS splits the data matrix into a new data structure as well as a residual matrix in which the noisy part of the data is gathered. The equation for the PCA decomposition is given by:

$$\begin{aligned} \boldsymbol{X} &= \boldsymbol{t_1 p_1}' + \boldsymbol{t_2 p_2}' + \ldots + \boldsymbol{t_a p_a}' + \boldsymbol{E} \\ \boldsymbol{X} &= \boldsymbol{T P}' + \boldsymbol{E} \end{aligned} \tag{4.6}$$

where $\mathbf{t_i}$ is the i:t principal component score vector, $\mathbf{p_i}$ is the i:t principal component loading vector, $\mathbf{T}$ is the score matrix and $\mathbf{P}$ the loading matrix and $\mathbf{E}$ the residual matrix.

As one can deduce from the formula above the number of principal components one can construct is equal to the smallest dimension of $\mathbf{X}$. Undoubtedly one does not need to use all these principal components and doing so would only be futile since this would mean that the data is represented in a new coordinate system but without any data nor noise reduction. The first principal component will describe the largest part of the variance in $\mathbf{X}$ and in a descending order afterwards each principal component will capture less and less variation and thus less information. There will be an optimal or close to optimal number of principal components to include in the model [7, 8].

4.6 Partial least square regression (PLS)

PLS is a short form for projection of latent structures by means of partial least squares [8]. In multivariate regression we are not only dealing with a data matrix $\mathbf{X}$ of the size $n \times k$, as used in PCA, a response data matrix $\mathbf{Y}$ of size $n \times m$ is added, where m is the number of corresponding response variables whom should be investigate for correlation to the given measured observation, to find relation between $\mathbf{X}$ and $\mathbf{Y}$.

The easiest way to look at PLS is that two PCA-resembling models are fitted to the $\mathbf{X}$ and $\mathbf{Y}$ matrices respectively. This is not completely true since PLS modelling uses the appearance of the $\mathbf{Y}$ matrix to influence the principal components of $\mathbf{X}$ and hence adapt it to the variance pattern found in $\mathbf{Y}$. This means that the variance captured by the principal components do not necessarily decrease in an orderly fashion as was the case in PCA. The PLS-algorithm NIPALS uses an extra loading weight $\mathbf{W}$, which directly connects to the building relationship of the $\mathbf{X}$ and $\mathbf{Y}$. As long as the dominant structures in $\mathbf{X}$ agree with the maximum direction of correlation in $\mathbf{Y}$, the loadings $\mathbf{P}$ and $\mathbf{W}$ remain similar. Should they show significant difference this would imply that the features found in $\mathbf{Y}$ do not correlate well to the dominant characteristics in $\mathbf{X}$ [7]. The formula for the decomposition of the two data matrices with the NIPALS algorithm are given below:

$$\boldsymbol{X} = \boldsymbol{T}\boldsymbol{P}' + \boldsymbol{E} \tag{4.7}$$

$$\boldsymbol{Y} = \boldsymbol{U}\boldsymbol{Q}' + \boldsymbol{F} \tag{4.8}$$

$$\widehat{\boldsymbol{Y}} = \left(\boldsymbol{W}(\boldsymbol{P}'\boldsymbol{W})^{-1}\boldsymbol{Q}'\right)\boldsymbol{X} \tag{4.9}$$

where $\mathbf{T}$, $\mathbf{U}$ are the score matrices and $\mathbf{P}$, $\mathbf{W}$, $\mathbf{Q}$ the loading matrices and $\mathbf{E}$, $\mathbf{F}$ the residual matrices for $\mathbf{X}$ and $\mathbf{Y}$ respectively. $\widehat{\mathbf{Y}}$ stands for the predicted value from a PLS model. To summarize PLS tries to capture the most variance within each data matrix $\mathbf{X}$ and $\mathbf{Y}$ and also take into account that the correlation between the two should be as large as possible.

One of the crucial points of PLS modelling is finding the optimal number of PLS components to include in the model. The maximal number of PLS components that can be constructed are the minimum dimension spanned by the X matrix, which is exactly of the same dimensions as in PCA, i.e.:

$$\text{Absolute number of principal components} = min(n, k) \tag{4.10}$$

The amount of variance captured by the PLS components will gradually increase and using all components would describe the total variation in both $\mathbf{X}$ and $\mathbf{Y}$. Although the model now can account for the total variation in both the data blocks this does not guarantee an accurate model, rather a grossly over-fitted model would be produced. One needs to differ between the model's fit and the model's predictability.

The question is how to find the optimal number of PLS components to accommodate the best possible predictability of the model. There are numerous techniques on how to achieve this, but the one most spread is the one of cross-validation described by Wold and Eriksson 1995 [8]. The cross-validation will *iteratively* calculate a value for the closeness of the fit for the model, called Q^2. Calculated in the following way:

$$Q^2 = 1 - \frac{\sum_{i=1}^{n} (y_i - \widehat{y}_{ip})^2}{\sum_{i=1}^{n} (y_i - \overline{y})^2} \tag{4.11}$$

where y_i are the observed response values and $\widehat{y}_{ip}$ the predicted response values obtained through cross-validation using only the p:th PLS component and $\overline{y}$ being the mean of all the measured responses. Hence we obtain a Q^2 for each PLS component revealing its individual significance. If $\widehat{y}_{ip}$ is calculated when all significant p PLS components are incorperated, a cumulative score for Q^2, called Q^2_{cum} is attained through the equation above. A Q^2_{cum} of 0.5 is considered to be good and a value of 0.9 is excellent. However, this value is very application specific, depending on what process is modelled. Hence a lower or higher Q^2_{cum} can be considered good or excellent.The explained variance of $\mathbf{Y}$, R^2Y_{cum}, will now be compared with Q^2_{cum}. If R^2Y_{cum} is much higher than Q^2_{cum} this implies that the model is suffering from an over-fit and thus the number of PLS components should be reduced.

A very short introduction to the science of multivariate data analysis has been presented here and the interested reader may therefore want to read some of the given references for further information on the subject [7, 8].

4.7 Useful tools in PCA/PLS

- Model error;

$$Model\ error = \mathbf{Y} - \widehat{\mathbf{Y}} \tag{4.12}$$

where $\widehat{\mathbf{Y}}$ is the estimated value of $\mathbf{Y}$, which is the true measured value.

- Validation Residual;

$$Residual\ Variation_{(i=cal.,\ val.\ or\ crossval.)} = \frac{\sum_{i=1}^{n}(y_i - \widehat{y}_i)^2}{n} \tag{4.13}$$

- RMSEC (Root mean square error of calibration);

$$RMSEC = \sqrt{residualvariation_{calibration}} \tag{4.14}$$

A measure of the error estimation for the calibration set.

- RMSEP (Root mean square error of prediction);

$$RMSEP = \sqrt{residualvariation_{prediction}} \tag{4.15}$$

A measure of the error estimation for the prediction of an external test set.

- RMSECV (Root mean square error of cross validation);

$$RMSECV = \sqrt{residualvariation_{crossvalidation}} \tag{4.16}$$

A measure of the error estimation when applying cross validation.

- Hotelling, T^2, is a measure of distance for each sample from the origin of the data set. With the help of this analytical tool one can distinguish observation that lie abnormally far away from the rest of the samples and hence might be considered to be outliers.

- $Q_{residual}$ is the distance from one point to the plane spanned by the principal or PLS components.

- Cross validation through permutation of the response matrix $\mathbf{Y}$. New values for R^2Y_{cum} and Q^2_{cum} are calculated and if these reveal a better model than for the original response matrix, this is a good indication of over-fit. By implementing the permutation it is possible to clarify the statistical validity of the calculated model.

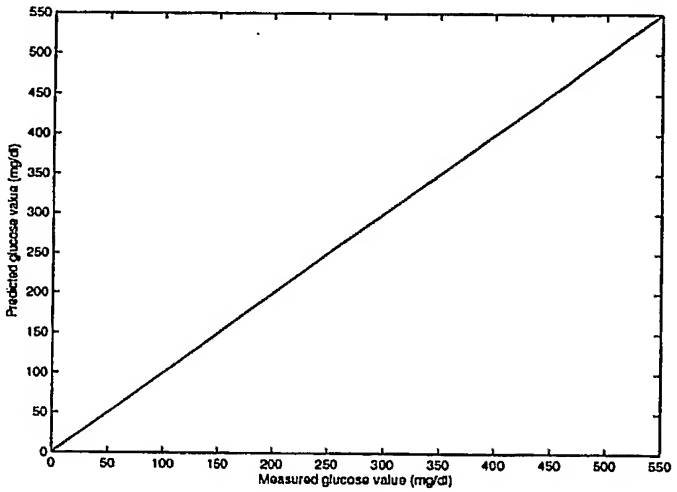

Figure 4.4: Standard x-y plot for presenting blood glucose measurements

4.8 Error grids

Results from measurements can be presented in many different ways. In this kind of measurements, where one measured value is used to predict a measured reference value, it is most common to create a plot like the one in fig 4.4, with the reference value on the X-axis and the predicted value on the Y-axis.

If the prediction of blood glucose level is perfect, all points will lie on the straight line in the middle ($y = x$). This is not especially common however and therefore a method for quantifying the errors is needed. The most obvious solution would be to use the same methods that are used in statistics (e.g. mean values and standard deviations). This is not particularly good for these kinds of predictions however, since the clinical consequence of the errors not only depends on the size of the error, but also on the absolute value of both the reference value and the predicted value. Some kind of error analysis that takes clinical information into consideration is needed, for instance the use of error grids (EG).

The use of EGs for error analysis of blood glucose measurements was first introduced by Clarke et al. [24] in 1987. The EG they constructed (fig 4.5), divides the plot into five risk categories:

A: Predicted value deviates less than 20% from the reference value, everything all right.

B: Predicted value deviates more than 20% from the reference value, but no treatment is needed.

C: The predicted values deviate a lot from an acceptable reference value and overcorrecting treatment may be the result.

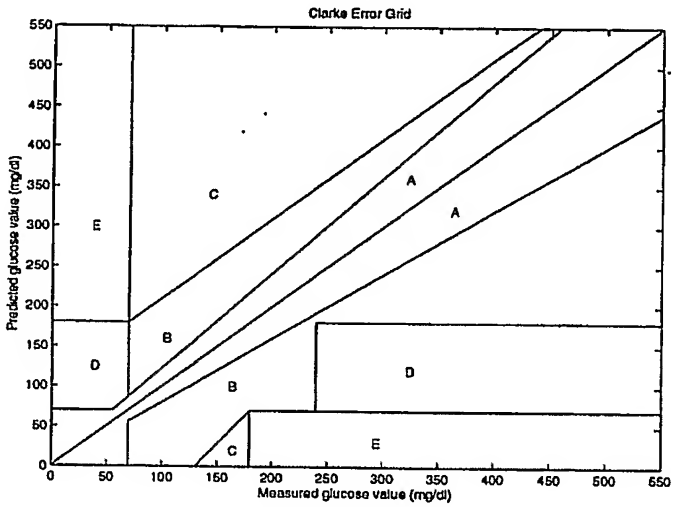

Figure 4.5: Clarke Error Grid

D: The predicted values show acceptable values, but in fact treatment is needed.

E: The predicted values will suggests treatment that is opposite to the needed treatment. These errors are potentially lethal for the patient.

Discussions about this EG lead to another publication, clarifying how the error grid should be used [25]. The most important comment in the article is that the prediction method *only* can be considered to be properly working when the values fall into category A. For *all* the other cases, the method needs further development, although values that fall into B do not endanger the patient.

A couple of years ago, a new kind of EG was presented by Parkes et al. [26], usually referred to as BD-grid. The new grid was introduced since their research group found the Clarke-grid unsatisfactory. Two of their remarks were that Clarke et al. only consulted a few clinicians when developing their EG and that the Clarke-grid had very sharp boundaries for the different risk categories. In the Clarke-grid two values that only differ slightly from each other (i.e. 200,71 and 200,69) end up in two severely different categories, the first one in category B (not dangerous) and the second in category E (very dangerous). Although both are unacceptable, the problem is that the different risk classifications will lead to severely different conclusions, a problem that is solved in the BD-grid.

The new EG was constructed based on a survey performed among 100 endocrinologists who treat diabetes patients. Each endocrinologist was asked to fill out a questionnaire, where they categorized the clinical risk of a number of BG measurements into one of five categories:

A: No effect on clinical action

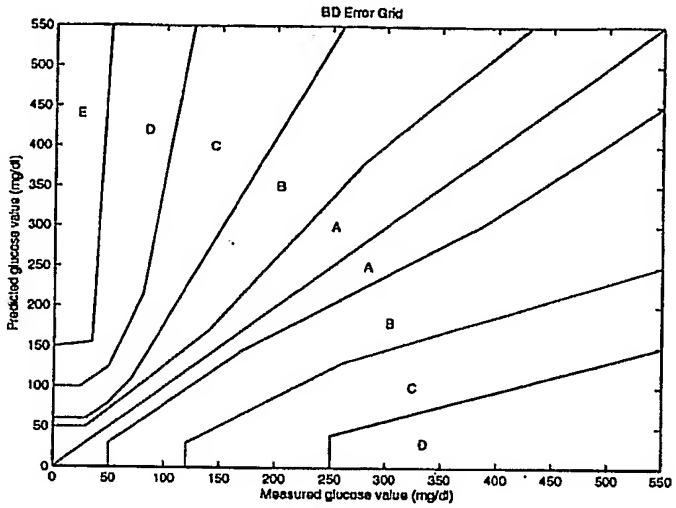

Figure 4.6: BD Error Grid

B: Altered clinical action or little or no effect on clinical outcome

C: Altered clinical action - likely to effect clinical outcome

D: Altered clinical action - could have significant medical risk

E: Altered clinical action - could have dangerous consequences

All the answers were then put together using arithmetic averaging, after assigning each risk a value between 0 and 4. Since this gave very uneven boundaries for the risk categories, the boundaries were smoothed with a filtering algorithm. The resulting EG is presented in fig 4.6.

In fact, Parkes et al. constructed two different EGs using the same methods, one for type 1 patients and one for type 2. Both EGs are quite similar, but not identical. However, since only diabetes type 1 is considered in this project, the second EG has been left out.

Which EG should be used then, Clarke or BD? The fact that BD is more or less an updated version of the Clarke-grid and that much more clinicians had an influence on the development, tipped the scale in this project. We will use the BD error grid.

One last remark though. Even with the use of EGs, mathematics can not be completely forgotten. The EG is a tool used to evaluate the *clinical* significance of an error. Statistical methods are still needed to express the overall relevancy of the prediction method. When only using EG analysis and little or no statistical analysis, one might get "lucky" and find all data within the A-category and then draw completely wrong conclusions anyway.

Chapter 5

Experiments

5.1 Basic experiments in saline solution

5.1.1 Introduction

Glucose is a true non-electrolyte, thus difficult to measure with electrical methods [5]. However, there is a connection between glucose level and skin bioimpedance, as shown by Harry Elden [33]. In order to understand that connection, one must start from the beginning, and investigate the link between glucose in a solution and impedance.

Given that the human body consists of approximately 70 % water, we decided to perform our basic experiment in a physiological saline solution, thereby investigating the existence of a link between glucose and impedance and on the same time take the first step towards *in vivo* measurements.

5.1.2 Materials and Methods

Setup

The saline solution was poured into a plastic container, having the length 11.5 cm, width 11.5 cm and height 11 cm. To this solution, water-free glucose powder was added. The saline solution and the glucose powder were measured to the correct amounts using a scale (AND EK-1200A). To decrease the solving time, a magnetic stirrer (Ikamag Ret-G) was used. Four brass electrodes were inserted, with a mutual distance of 3 cm to which an impedance meter (Fluke PM6304) was connected.

Experimental method

In order to simulate different glucose concentrations in ECF, the glucose concentration in the saline solution was gradually increased. Impedance measurements were performed with four-point technique at 14 different concentrations, with four different frequencies (0.1, 1, 10, 100 kHz), yielding a total of 56 measurements.

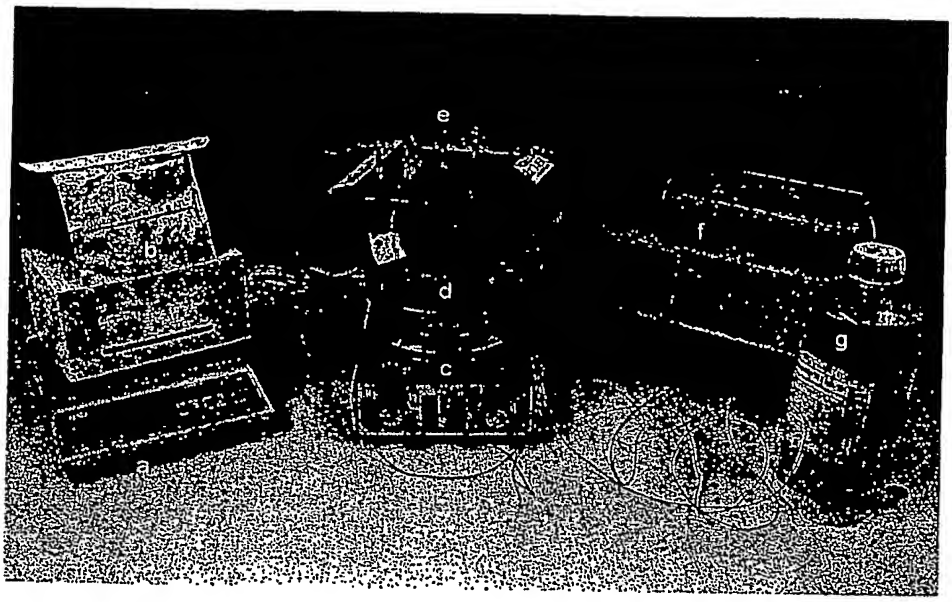

Figure 5.1: Equipment setup: a) Scale, b) Glucose powder, c) Magnetic Stirrer, d) Saline/Glucose solution, e) Brass electrodes, f) RCL-meter, g) Saline solution

Numerical

To make it easier to see possible trends in the data, impedances values were plotted vs. glucose concentration in a two-dimensional Cartesian space. A line was fitted to the data using the least square method. Since all measurements suffer from errors in some degree, error bars were used to illustrate this occurrence.

5.1.3 Results

From the experiment described above, a clear link was found between glucose level and the impedance value, as seen in figure 5.2. The figure shows the results at 100 Hz, but the plot looks almost identical for all four frequencies.

Usually one would present resistivity instead of the impedance magnitude, but that is quite hard to do in this case. In order to calculate the resisitvity from the impedance, advanced computer programs are needed, because of the geometry of the electrodes and the plastic container. Since we are only interested in finding out if changing the glucose concentration will yield an electrically measurable change, the impedance magnitude will work fine.

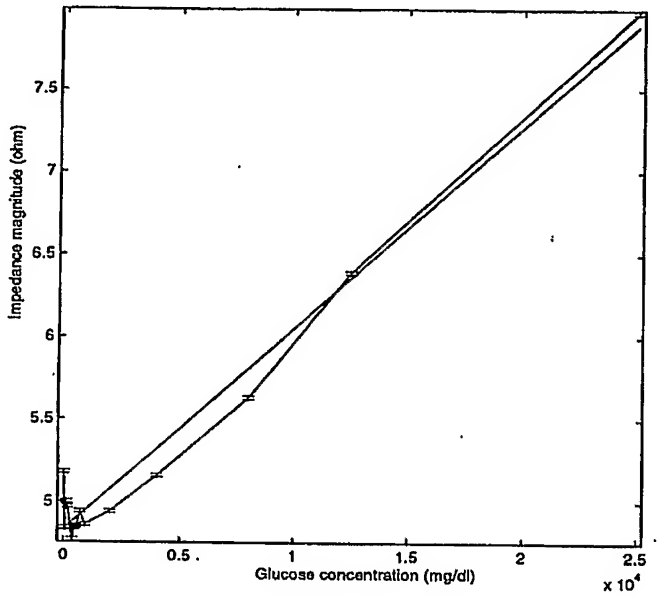

Figure 5.2: Impedance values vs. glucose concentration in the saline solution at 100 Hz.

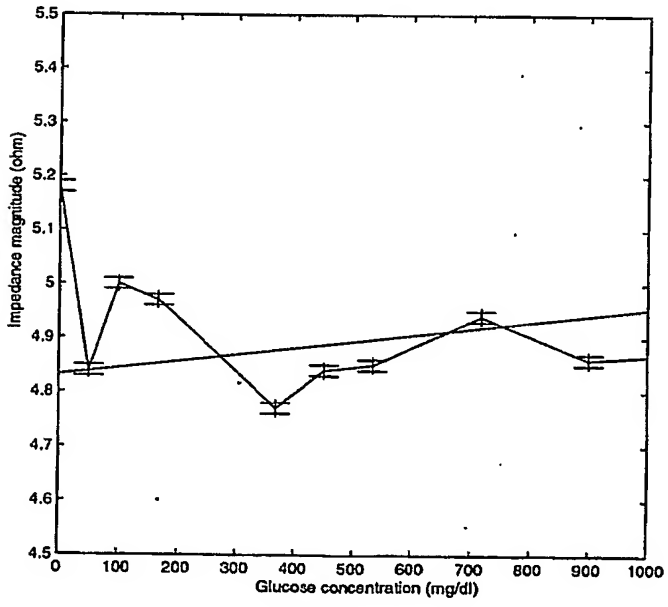

Figure 5.3: A closer look at the impedance in the physiological interval.

5.1.4 Discussion

Although there exists a clear trend between impedance and glucose, the changes occurring in the physiological interval (see chapter 2, "Medical Background") is rather small or even non-significant. Clearly the values are fluctuating in a non-orderly fashion. As illustrated by the error bars, the error is quite big in this interval, and consequently the possibility of a trend still exists.

The observed increase of impedance magnitude might be explained by the fact that the viscosity of the solution will be increased as the glucose concentration is raised. According to Walden's rule [11], the ion mobility will be decreased as the viscosity is increased, at least in an infinitely diluted solution, but it should be a good approximation in this case as well.

5.1.5 Conclusion

The conclusion that must be drawn is that there exists a correlation between glucose and impedance. Two questions still remain unanswered though:

- Can the correlation be observed within a cell structure?
- Is there really a trend in the interesting interval?

5.1.6 Further experiments

Another experiment should be conducted with a better model of human tissue, which takes the cell structure into consideration.

5.2 Basic experiments with saline solution in oasis

5.2.1 Introduction

In the first experiment, the human body was simulated using physiological saline solution. As we all know, there is more to the human body than just salt water. The purpose of the first experiment was to establish a link between impedance and glucose content in a solution. Now this link is taken one step further towards *in vivo* measurements, by building a better model of the human body.

5.2.2 Materials and Methods

Setup

This experiment resembles the first one, but with greater consideration taken to the simulation of tissue. Saline solution works just fine as a simulation of ECF, but it does not consider the cellular component. To improve our simulation of tissue, oasis blocks soaked with a solution of physiological saline and glucose were used. Of course the cellular structure of oasis is not a perfect model of the human cell structure, but it is a far better model than just salt water.

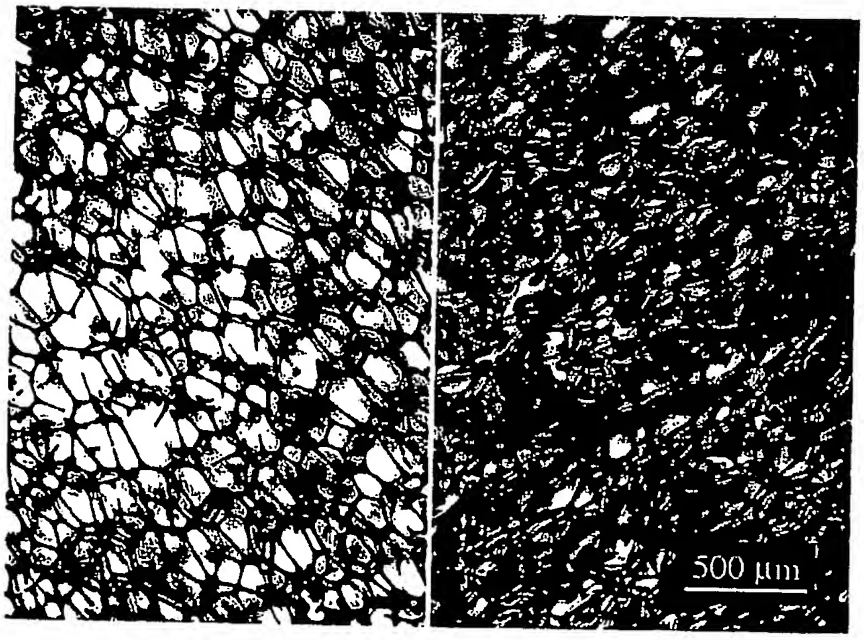

Figure 5.4: Microscopic picture of an oasis block. Left: Ordinary cell structure. Right: Cell structure soaked with water. Clearly the cell structure has been affected by the water.

The blocks were standardized to following sizes: length 11 cm, width 7.5 cm

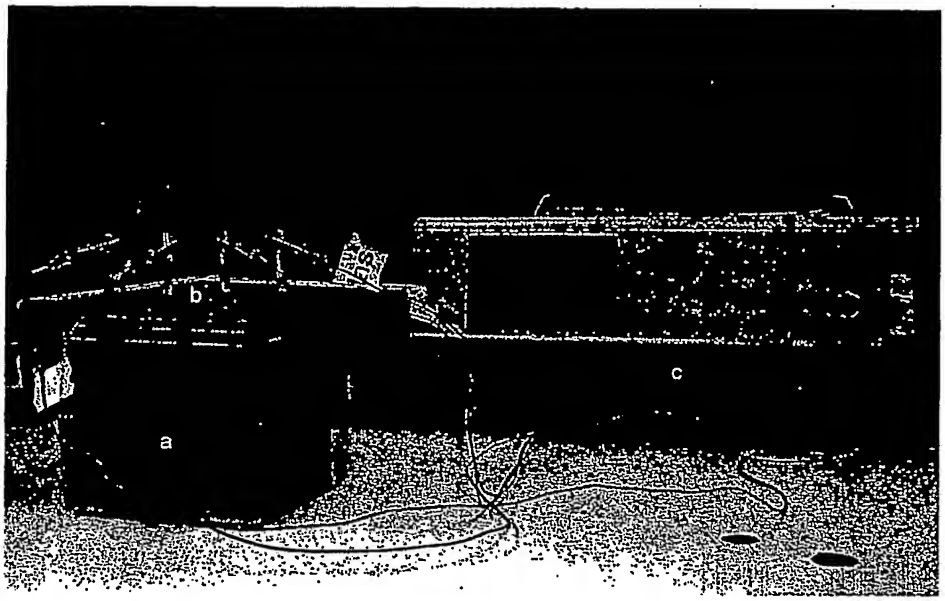

Figure 5.5: Experimental setup: a) Oasis block, b) Electrodes and c) RCL-meter

and height 8 cm. These were then placed inside a plastic container and four brass electrodes were inserted at a mutual distance of 3 cm.

Experimental procedure

In order to simulate different concentrations of glucose within tissue, solutions with different concentrations of glucose were prepared before being added to the oasis blocks. To make certain that the oasis block had the given concentration, a new block of oasis was used for each measurement. Impedance measurements were performed with four-point technique for 6 different concentrations, at four different frequencies (0.1, 1, 10, 100 kHz), yielding a total of 24 measurements.

After the first measurement series, it turned out that some of the oasis blocks were dry in the middle. This meant that these measurements had to be discarded and redone. After each following measurement the oasis blocks were cut in half and the values were stored only if they were found to be soaked.

Numerical

To make it easier to see possible trends in the data, impedances values were plotted vs. glucose concentration in an ordinary Cartesian plot. A line was fitted to the data using the least square method. Since all measurements suffer from errors in some degree, error bars were used to illustrate this occurrence.

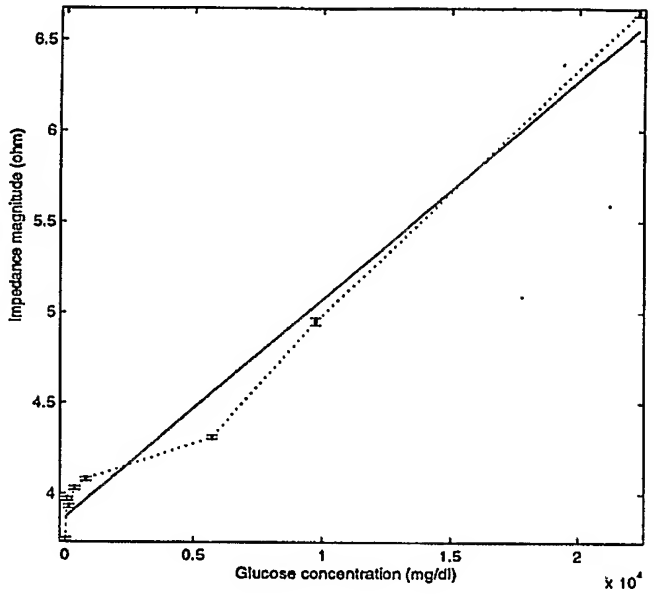

Figure 5.6: Impedance values vs. glucose concentration in the oasis blocks, measured at 100 Hz

5.2.3 Results

From the experiment described above, a clear link was found between glucose level and the impedance value, as seen in figure 5.6. The figure shows the results at 100 Hz, but the plot looks almost identical for all four frequencies.

5.2.4 Discussion

There exists a trend between impedance and glucose in the cell structure. In the physiological interval there seems to be an even stronger effect of the glucose concentration upon the impedance value. Obviously at least twice as many measurements are needed to be certain of the trend. Given that glucose is a true non-electrolyte [5] the observed trend could be a consequence of increased viscosity, which would increase the impedance. It could also be explained by the fact that the current has to travel a longer distance between the electrodes.

Comparing this experiment with the one conducted without the oasis block shows a change in the base impedance value. This is of no concern since the measurements are conducted in slightly different volumes and thereby the size of the electrode in contact with the measurement object will be slightly different. This will of course affect the impedance value. If both measurements had been conducted in exactly the same volume, the impedance value would most likely differ less.

5.2.5 Conclusion

The conclusion is that there exists a correlation between glucose and impedance in a cell structure too, not only in the saline solution. The question still remains if the correlation between glucose and impedance can be observed *in vivo*.

5.2.6 Further experiments

For future measurements the construction of an even better model of tissue should be considered. There are at least two ways of constructing such a model. One would be to find a material with a cell structure even more resembling human tissue. The other way would be to construct capillaries in the cell structure. This would simulate the blood vessels of the dermis and one could observe the diffusion of the glucose into the structure.

5.3 Completely non-invasive measurements

5.3.1 Introduction

It has been shown that an increase of glucose concentration in a saline solution increases the impedance. The same behaviour can be found in an open cell structure (e.g. oasis) soaked with a saline/glucose solution.

From previous experiments in the U.S., it is known that skin impedance is correlated to the blood glucose level [33, 34]. In some subjects the correlation is quite good, but in other subjects it is hard to find. Furthermore the correlation diminishes over time and therefore this method is not yet applicable in a clinical device for monitoring of the blood glucose level.

The first aim of this experiment is to try to establish such a correlation in two subjects and the second to investigate different factors that will affect the measurable signals and hence the correlation.

5.3.2 Materials and Methods

Setup

Skin impedance was measured using the SciBase II skin impedance spectrometer (chapter 3, section 3.3). To increase the conductance of the horny layer, it was moisturized with physiological saline solution. In this experiment the volar forearms of two volunteers were used. One volunteer is diagnosed with Diabetes Mellitus type 1, whilst the other subject is not known to suffer from neither Diabetes nor any other blood or skin related diseases. Impedance was measured every ten minutes for approximately 4 hours during several days.

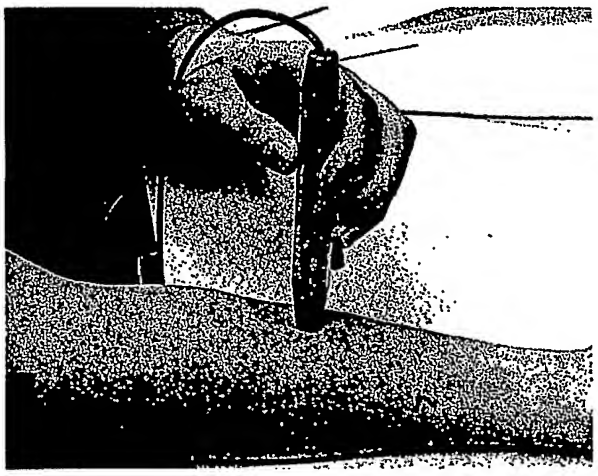

Figure 5.7: The probe of the SciBase II applied on the volar forearm of one subject.

Experimental procedure

On the volar forearms of the volunteers, two hairless sites measuring 2x2 cm, located above and below the middle of the arm, were marked. One site at a time was moistened with saline solution during 60 seconds, using a paper tissue with the dimensions 4.5x6.5 cm soaked with 2.5 ml of saline solution. Quickly afterwards excess water was wiped off and the probe was placed on the marked site. The probe rested on the skin with merely its own weight for ten seconds in order to establish a good contact. Skin measurements were then carried out with the impedance spectrometer at five different depth settings using 31 frequencies for each depth. This resulted in 155 magnitude and 155 phase values after a measuring time of 20 seconds.

Numerical

The data acquired were studied using two different approaches. The first was to correlate blood glucose against four different indices established by Ollmar and Nicander [18].

$$
\begin{aligned}
MIX &= \frac{|Z_{20\text{kHz}}|}{|Z_{500\text{kHz}}|} \\
PIX &= \arg(Z_{20\text{kHz}}) - \arg(Z_{500\text{kHz}}) \\
RIX &= \frac{Re\{Z_{20\text{kHZ}}\}}{|Z_{500\text{kHz}}|} \\
IMIX &= \frac{Im\{Z_{20\text{kHZ}}\}}{|Z_{500\text{kHz}}|}
\end{aligned}
$$

Secondly the data acquired was analysed using PCA to show possible outliers or drifts within the measured data. PLS was then applied to the data in order to correlate the impedance against blood glucose measured in a drop of blood from a fingertip.

5.3.3 Results

For the non-diabetic subject, the data showed significant fluctuations between consecutive measurements even without changes in blood glucose level. The fluctuations were so severe that the hope of finding any correlation was close to non-existent. Therefore the data was discarded and the results presented below originate only from the diabetic subject.

From the four indices no apparent correlation could be established (fig 5.8). The indices have shown significance in other applications, such as quantification and classification of skin irritation [14]. The fact that the indices did not show any apparent correlation in this experiment illustrates that they are application specific. More advanced data analysis could possibly reveal more subtle trends in the data.

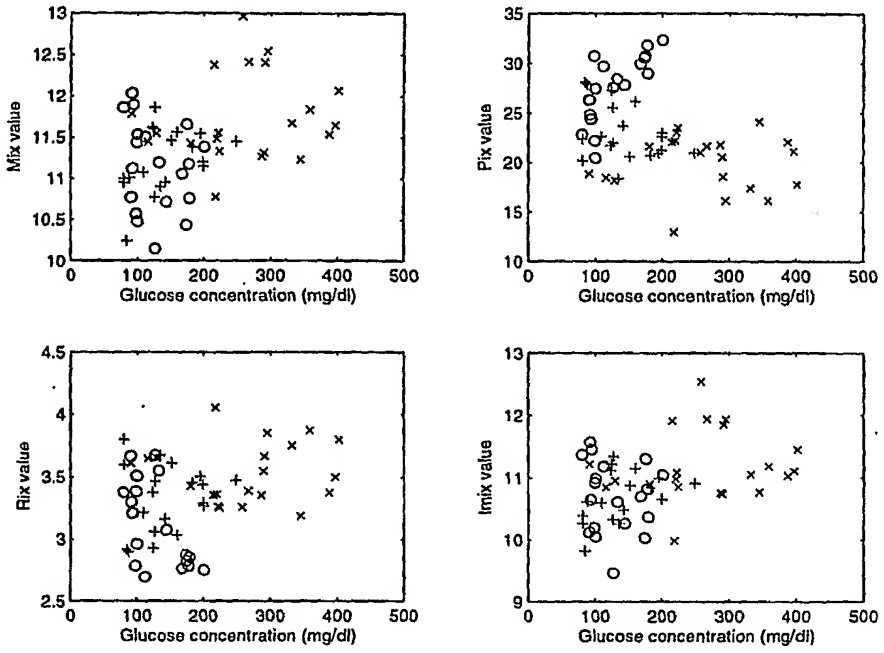

Figure 5.8: Impedance indices plotted vs. glucose levels. Only depth setting 3 was used, since all depth settings showed similar patterns. The different symbols represent different days, o = 9 sept, + = 11 sept and × = 30 sept.

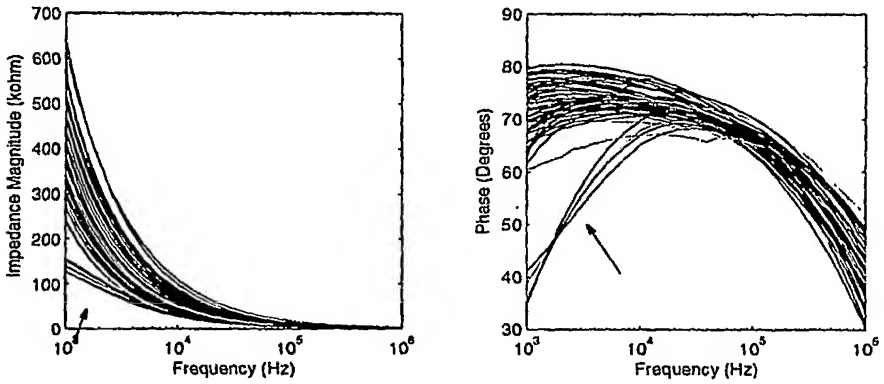

Figure 5.9: Raw data of the first depth setting. Possible outliers marked with arrow.

Before any analysis was applied, the data was plotted in a raw data plot. Figure 5.9 shows only raw data for the first of five depths, since all depths were almost identical. Note that some magnitude and phase curves are fairly separated from the rest.

Applying PCA on the raw data quickly showed the existence of four outliers in the score plot (fig 5.10). These outliers correspond to the magnitude and phase curves marked in the raw data plot (fig 5.9). They are probably the result of either too much inundation, excess fluid on the skin surface or long time storage of fluid in the top layers of the skin.

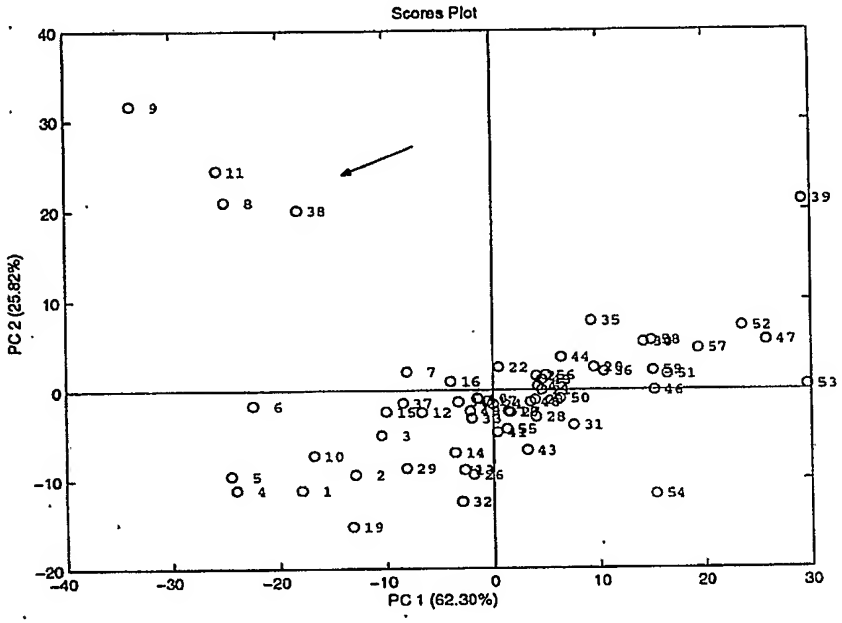

Figure 5.10: Scoreplot from PCA model. Outliers marked with an arrow.

After removing these outliers from the data, PCA was applied once more. No new critical outliers were found and the remaining data are considered to be a good base for further analysis. However, the new score plot indicated that the different days were separated from each other. To visualize this more clearly, a box plot of the information in the score plot was created (see fig 5.11). This shows a significant drift in the measurements between the days, probably due to structural changes in the skin.

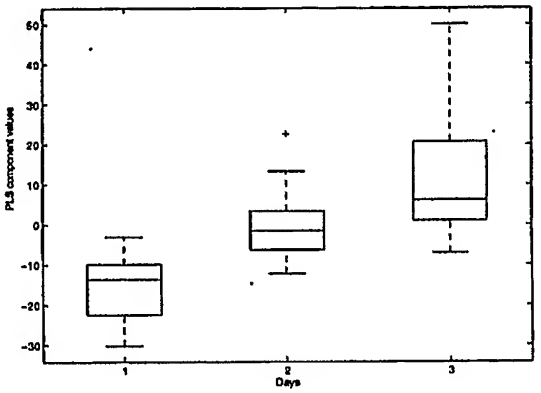

Figure 5.11: Boxplot of the first PC of the PCA model, showing significant separation between days.

An attempt was made to rid the data of this base shift, by using following manipulation:

$$x_i = \frac{x_i - \overline{x}}{std(x)} \tag{5.1}$$

The result of this manipulation was unfortunately not sufficient.

Although there still remained a drift in the data, a PLS model was created using the impedance and glucose data. The first PLS component of this model captured approximately 97% (table 5.1) of the variance in the impedance data, while only explaining 15% of the glucose variation. The following two PLS only captured around 1% each of the impedance variance, but 25% and 3% of the glucose variance respectively. However they still gave a lowered RMSECV-value (fig 5.12), which would indicate that they should be included. Even if three components are used R^2Y_{cum} remains low and Q^2_{cum} insignificant. Hence this must be considered to be a poor model.

Table 5.1: Variation captured in the x- and y-block for the first three PLS components.

	X-block		Y-block	
Comp. No.	This comp.	Total	This comp.	Total
1	96.94	96.94	15.20	15.20
2	0.48	97.43	25.15	40.35
3	1.01	98.44	2.67	43.01

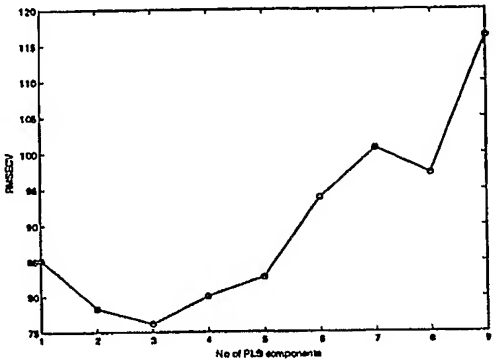

Figure 5.12: RMSECV values for increasing number of PLS components.

A number of different preprocessing steps were applied to try to improve the model:

- **Logging the magnitudes:** The impedance magnitude has a skew distribution that will affect the PLS model. To come to terms with this the magnitudes were logged, to give a more normal distribution.
- **Complex conversion:** The magnitude and phase data were converted to real and imaginary parts in order to investigate if a correlation could be found in either the real or imaginary part.
- **Logging complex converted data:** The complex converted data were logged to yield a more normal distribution as described above.
- **Statistical methods on complex data:** Eq. (5.1) was used again, this time applied on the complex converted data.

None of these manipulations improved the model significantly.

5.3.4 Discussion

In previous measurements, attempts have been made to come to terms with the drift described above. One of the problems is that the skin is at constant change, a change that will give rise to errors in a static model. Of course one could construct a dynamic model instead, if all the variables were known. It would be even better to create a static model where all uninteresting factors were removed.

Since the impedance of the horny layer has a major influence on the impedance spectra, the environmental effects acting upon this layer are of utmost interest. One of these is the relative humidity, as it will affect the moisture in the horny layer and in turn change the impedance of the skin. Different chemicals have also

shown to give significant changes in the skin [14]. Every day the skin is in contact with chemicals, for instance through pollution or personal hygiene products.

One approach to improve the model could be to establish a calibration value for each individual and then calibrate the measurements according to this value every day. Nonetheless, not all the errors can be controlled by calibration. For instance a change of hygiene products might change the impedance spectra drastically and then a new calibration value has to be established.

If the calibration approach is not effective, another approach would be to exclude the horny layer from the measurements and thereby decreasing the influence of the environmental effects mentioned above. The classical way to achieve this would be to mechanically remove part of the horny layer and then repeat the measurements.

The question is then if this measurement principle is generally applicable or if it only works on some subjects. In this experiment, no correlation was detected in neither of the two subjects, but it has been previously shown to work on some subjects [34].

5.3.5 Conclusion

No clear correlation could be established between skin impedance and blood glucose concentration and hence no extensive investigation of the affecting parameters could be conducted.

5.3.6 Further experiments

Since no clear correlation could be generated from the measurements a new approach should be considered. The horny layer is undergoing the most severe changes and the mere fact that impedance of this layer is a dominant factor, one interesting approach would be to make an attempt of excluding it from the measurements.

Research in MEMS-technology (Micro Electro Mechanical Systems) [21] has resulted in a new approach to reduce the effect of the horny layer from the measurements. Using a new probe based on this technology with the impedance spectrometer, might improve the measurements and will be used in the following experiment.

5.4 Minimally invasive measurement

5.4.1 Introduction

The completely non-invasive measurement gave no clear correlation between blood glucose and impedance in any of the two subjects. One suggested approach to improve the results was to reduce the effect of the horny layer. In order to experiment with this approach, a new probe is used together with the Scibase II, making it possible to measure inside the skin. The main goal is still to establish a correlation between blood glucose concentration and impedance.

5.4.2 Materials and Methods

Setup

Skin impedance was measured using the Scibase II impedance spectrometer with a new probe based on MEMS-technology. The head of the probe consists of a silicon plate with numerous microscopic spikes [21]. When applied to the skin, the spikes will penetrate the top layer of the skin and thereby reduce the impedance magnitude, since the horny layer has the most dominant dielectric influence on skin impedance (5.13).

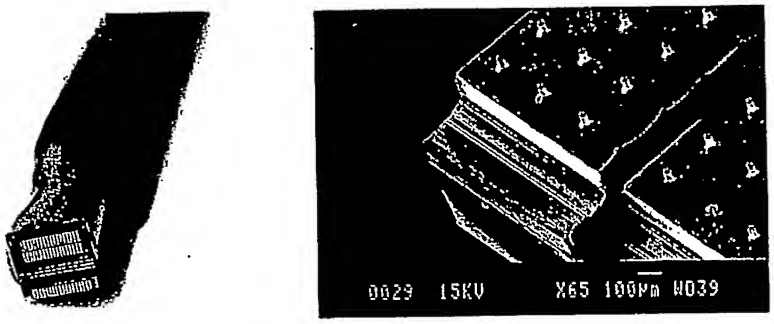

Figure 5.13: Photograph of a prototype spike probe with an EM picture of the spikes.

The measurements were conducted during the experiment "Invasive II" (described in section 5.6) in a shielded environment. The left volar forearm of one volunteer diagnosed with Diabetes Mellitus type 1 was used for the measurements.

Experimental procedure

New hairless sites on the volar forearm were selected for each consecutive measurement (fig 5.14 right), since the probe could possible cause irritation and oedema in the skin and thus distort the impedance spectrum. The selected site was moistened

with physiological saline solution in the same way as in the previous experiment (described in Experimental procedure, page 46).

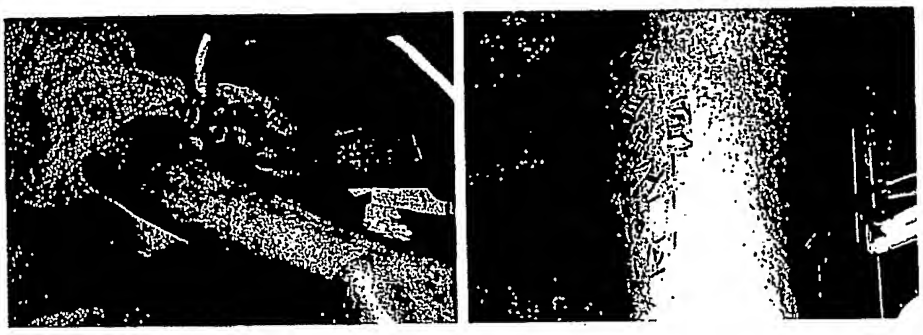

Figure 5.14: Photographs of the volar forearm of the subject. The left picture shows the probe pressed against the arm and the right shows the arm after the measurements, with all the measurement sites marked.

To assure that the spikes really penetrated the skin, the probe was pressed against the skin with moderate force during the entire sampling of one measurement (fig 5.14 left). As before, five depth settings were used and resulted in 310 values. The impedance measurements were conducted together with invasive glucose measurements (fig 5.25, page 64) every ten minutes for approximately 5 hours.

Numerical

Data from the measurements were first examined in a raw data plot and then in a PCA score plot to reveal possible outliers. After removing outliers the data was fitted to the measured glucose levels in a PLS model.

5.4.3 Results

The data was first examined in a raw data plot (fig 5.15), where a couple of measurements deviated substantially from the rest. Some measurements showed signs of interference. To further analyse the impedance data, a PCA score plot was created (fig 5.16 left). In the score plot, the measurements that deviated in the raw data plot were found to be outliers.

Some of the deviating points (28, 29 and 32) correspond to measurements that were conducted with different probes and were therefore excluded. The probe change was made since the measurements done with original probe started to become noisy, probably caused by probe degeneration. This degeneration was also the cause for the exclusion of the points 21, 26 and 27. After the exclusion of the outliers, a new PCA score plot (fig 5.16 right) revealed three new deviating points, but since these could not be explained by any obvious measurement error, they were not excluded.

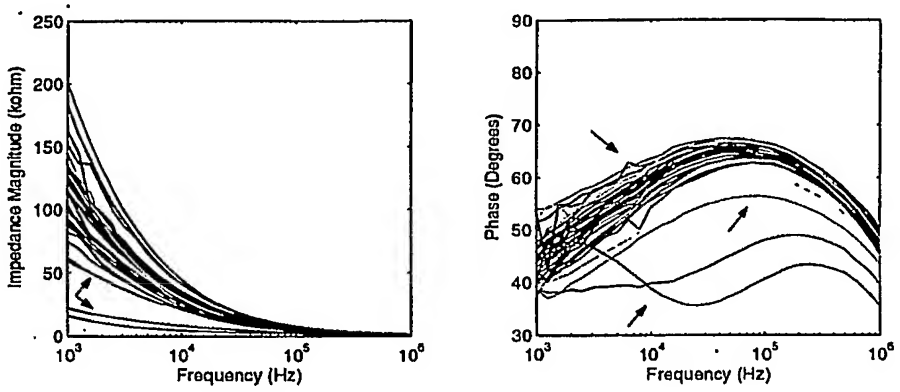

Figure 5.15: Raw data plot for only the first depth setting, since all depths showed similar appearance. Suspicious measurements marked with arrows.

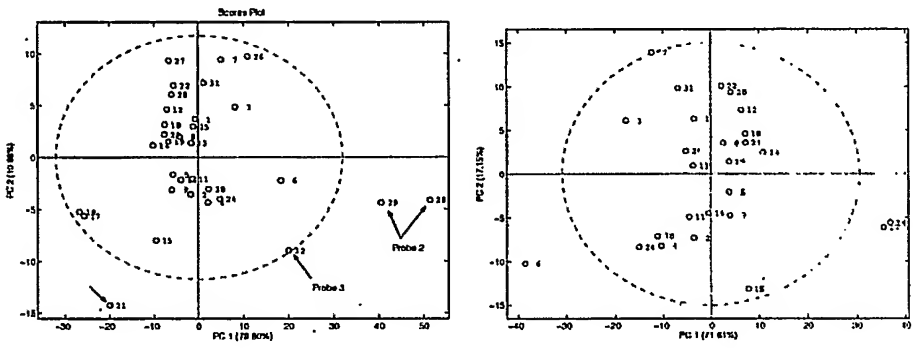

Figure 5.16: PCA Score plot before (left) and after (right) the exclusion of outliers marked with arrows in the left score plot.

A PLS model was constructed with the reduced data set, using the glucose measurements as reference. Cross validation was performed using SIMCA (Umetrics Software) and resulting values for R^2Y_{cum} and Q^2_{cum}, as well as RMSECV, are shown in figure (fig 5.17).

The RMSECV plot showed clear signs of overfit, if not already for the first PLS component then at least for the second PLS component and onward. This also reveals itself when looking on the Q^2s in fig 5.17, calculated for each PLS component individually.

Manipulations described in the previous experiment (page 50) were applied once more, but without any significant change in either R^2Y_{cum} or Q^2_{cum}.

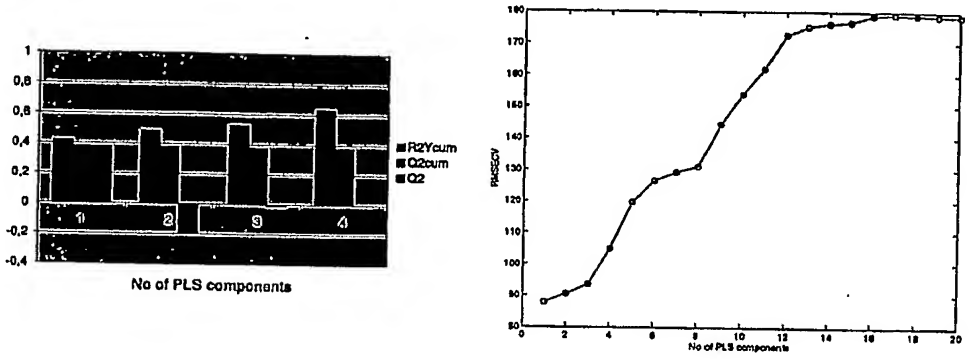

Figure 5.17: R^2Y_{cum}, Q^2_{cum} and Q^2 (left) and RMSECV (right) for increasing number of PLS components.

5.4.4 Discussion

No clear correlation between blood glucose concentration and skin impedance could be established in this experiment. Consecutive measurements seem to be randomly spread in the PCA score plot (fig 5.16) and not close to either their predecessor or successor. This is especially clear for samples 1 to 6, since these have similar blood glucose concentration.

There is many possible reasons for the fact that the data does not give any decent results. Since the measurements were conducted in a shielded environment with noise insensitive equipment, interference from the environment should not affect the measurements. Other possibilities for the poor data could be the new kind of probe or once again, the constant change of the skin (see chapter 5.3, page 50).

The theoretical base of the spike probe is quite interesting, since it gives a possibility to reduce the effect of the horny layer, without causing any pain and a minimum of skin irritation. However, the measurements conducted with the probe show signs of instability. The spikes could break easily because of their size ($\approx 30\mu m$ in diameter) and the bond wires of the prototype probe used in this experiment are placed in an exposed position on the sides of the chip, which makes them vulnerable to mechanical stress. The degeneration observed in the experiment may thus be caused by wear out symptoms of the probe.

To be able to establish a correlation between blood glucose and skin impedance, more accurate data is needed. This will probably be possible to achieve with an improved version of the probe, one that does not degenerate as easily. With more accurate data, as well as more data from more than one subject, it may be possible to establish a correlation that holds for one day at a time, since a correlation has been detected in earlier experiments on the skin surface [34]. Once such a correlation has been established, it will be of great interest to investigate the stability of this

correlation over time. The stability will most likely be improved compared to the regular probe, since the effect of the horny layer has been reduced and thereby the measurements will be less sensitive to the environmental factors discussed in the previous experiment (page 50).

5.4.5 Conclusion

This experiment did not reveal any correlation between blood glucose concentration and skin impedance. This is most likely due to the probe degeneration, as well as the skin change discussed earlier.

However, not enough data was gathered to truly dismiss the idea of finding a correlation inside the skin, using the spike probe.

5.4.6 Further experiments

Since no clear correlation has been established for the subject in either of the skin impedance experiments, it would be of great interest to examine if a correlation exists in other tissues within the body.

In order to determine if a correlation between skin impedance and glucose concentration exists, further experiments should be performed with an improved probe on multiple subjects.

5.5 Invasive measurement I

5.5.1 Introduction

In both the non-invasive and the minimally invasive experiments, a small correlation can be detected between glucose concentration and impedance magnitude. The fact that the correlation is stronger inside the skin suggested that a completely invasive measurement should be conducted to find out if the signal is even stronger in a muscle.

5.5.2 Materials and Methods

Setup

These measurements were carried out using an instrument called IBSA (Impedance Body Segment Analyzer). It is an impedance spectrometer just like the SciBase II, but it measures in the frequency range from 5 Hz to 500 kHz and it has been converted to 4-point measurements. Circuitry for separation of current injection and voltage detection has also been added. The instrument has been approved by the Department of Medical Technology at Huddinge University Hospital and fulfils the demands set by IEC 601-1:BF, and has been used in earlier studies on transplanted kidneys [16]. Since IBSA measures with 4-point technique (see chapter 3) four electrodes were used, with surface electrodes as the driving electrodes and needles as the sensing electrodes.

The measurements were performed in the right leg of one volunteer suffering from diabetes mellitus type 1. The positions of the needles were decided after studies of the human anatomy (see fig 5.18), as well as discussions with medical personnel. A Styrofoam form was used to stabilize the leg during the measurement period, since movement will affect the measurements (fig 5.19).

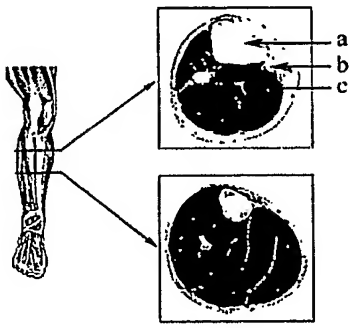

Figure 5.18: Cross section of a human leg, where a) is bone, b) adipose tissue and c) muscle tissue. *From: Virtual Hospital (www.vh.org) and Loyola University Medical Education Network (www.meddean.luc.edu/lumen)*

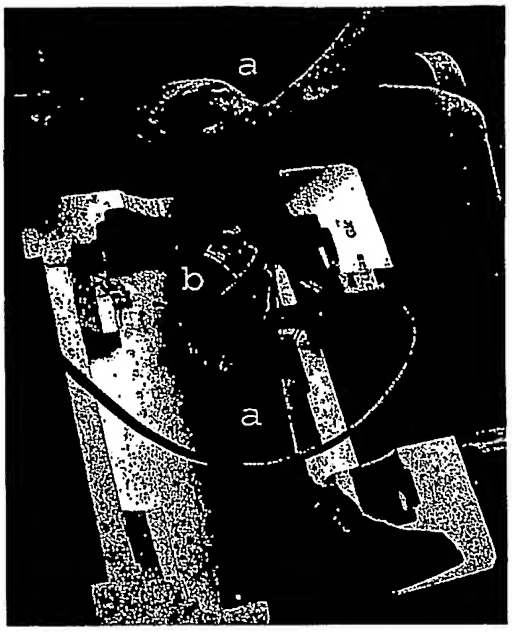

Figure 5.19: Photograph of the measurement setup. The surface electrodes are marked a) and the needles are marked b).

An estimated blood glucose value for each measurement was obtained using an invasive blood glucose meter (Glucometer Elite XL 3901E, Bayer).

Experimental procedure

The measurements started approximately 60 minutes after the needles were put into place, in order to ensure a minimum of remaining inflammatory responses [15].

Impedance values and blood glucose were registered every 10 minutes for roughly four hours. For each impedance measurement, five registrations were made to be able to investigate the deviation within each measurement compared to the deviation between consecutive ones.

To achieve a wide span of blood glucose concentrations in the subject, 75 g of water-free glucose powder dissolved in water were consumed after the first hour. When the blood glucose reached a high enough value, insulin was administered yielding a drop in the glucose value. Lunch was also consumed during the measurement period. A plot of the blood glucose values can be seen in figure 5.20.

At the end of the experiment the blood glucose of the subject was thoroughly checked for a longer time to make sure hypoglycaemia did not set in.

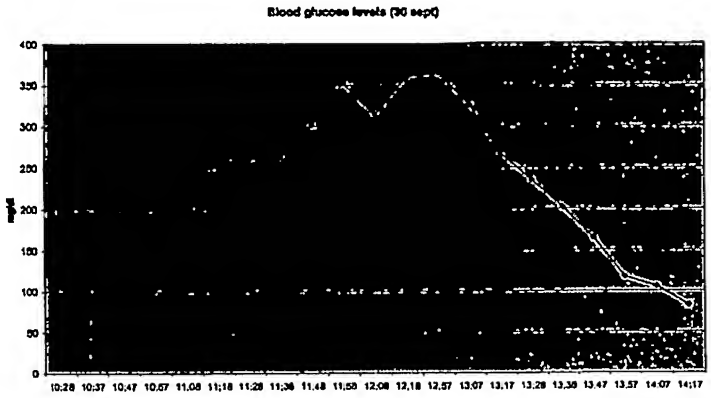

Figure 5.20: Blood glucose concentrations during the measurement.

Numerical

The collected data was analysed in several steps. First the raw data was visualized as is, without any data analysis applied. Thereafter statistical methods box plot, mean value and standard deviation were used to reveal artefacts such as noise. PCA was used to make sure that no outliers would affect the model (see chapter 4).

5.5.3 Results and Discussion

The raw data gathered from the measurements are shown in figure 5.21.

The data shows signs of interference at specific frequencies. Since all frequencies are cross-correlated, there should be no apparent discontinuities in the plot. Three distinctive frequency ranges are affected by noise (5-10 Hz, 1-2 kHz and 100-500 kHz. To examine this further, a box plot was made (fig 5.21). In this plot it is quite easy to see the base shift at approximately 100 kHz and the noise in some of the lower frequencies.

There may still be information of interest in the data although it is overshadowed by noise. The simplest way of dealing with this fact is to exclude these frequencies from the data set.

After those adjustments, the data set was analysed with PCA to assure that all five registrations that were done for each measurement showed similar characteristics. This was visualized with a score plot of the first two principal components (fig 5.22).

In the left figure, a score plot based on the first two principal components are shown. Since all five replicas for each measurement point are plotted it is evidently hard to separate the measurements from each other. In the right figure, each measurement is represented by one ellips. The center of each ellips represents the

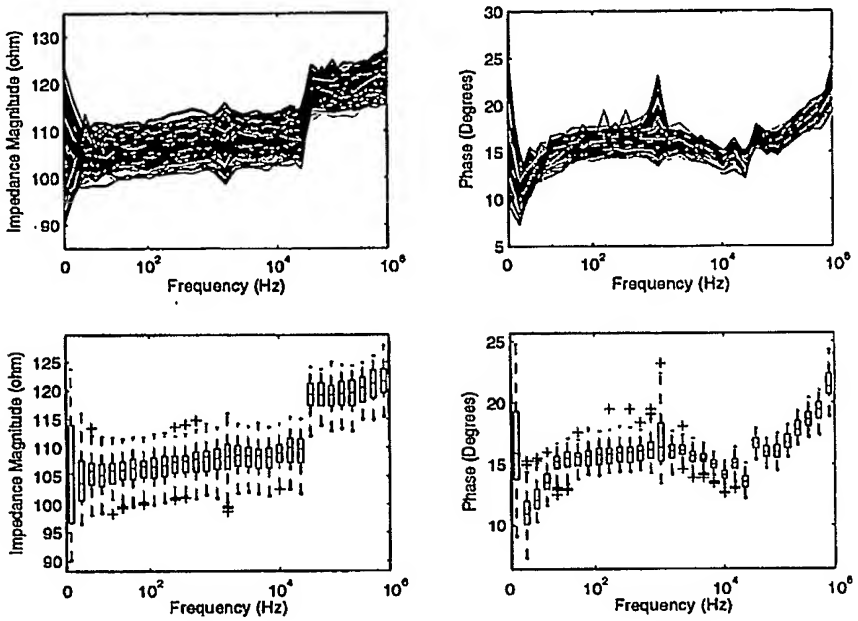

Figure 5.21: Raw data and boxplot of raw data.

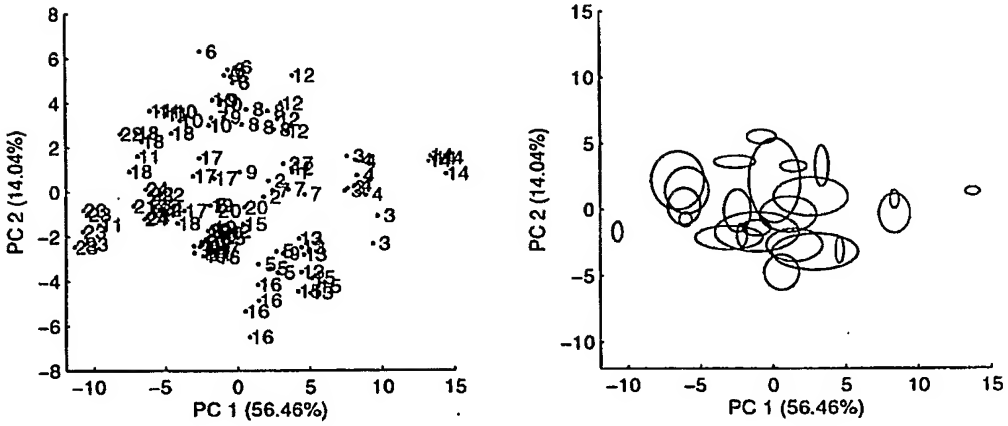

Figure 5.22: Left: Score plot of the two first principal components. Right: Modified score plot, where each ellips represents the five replicas of one measurement point.

mean value for that measurement. The x-radius and y-radius describes the standard deviations of the first and second principal components respectively. The fact that several ellipses have a large area, implies that the total deviation is large within one observation. This gives the conclusion that even though some frequencies were excluded, the data is still affected by noise.

Since each ellips represents one observation with five replicas it can also be seen that the variation within one observation often is bigger than the variation between different observations. This could result in problems when creating a model for glucose prediction, since there will not exist any clear one-to-one relation between glucose levels and impedance data.

The remaining noise has to be reduced in order to draw any conclusions. A possible approach could be to smoothen the data using for example the Savitzky Golay algorithm [8]. Unfortunately no remarkable improvements could be seen when a new PCA plot was made.

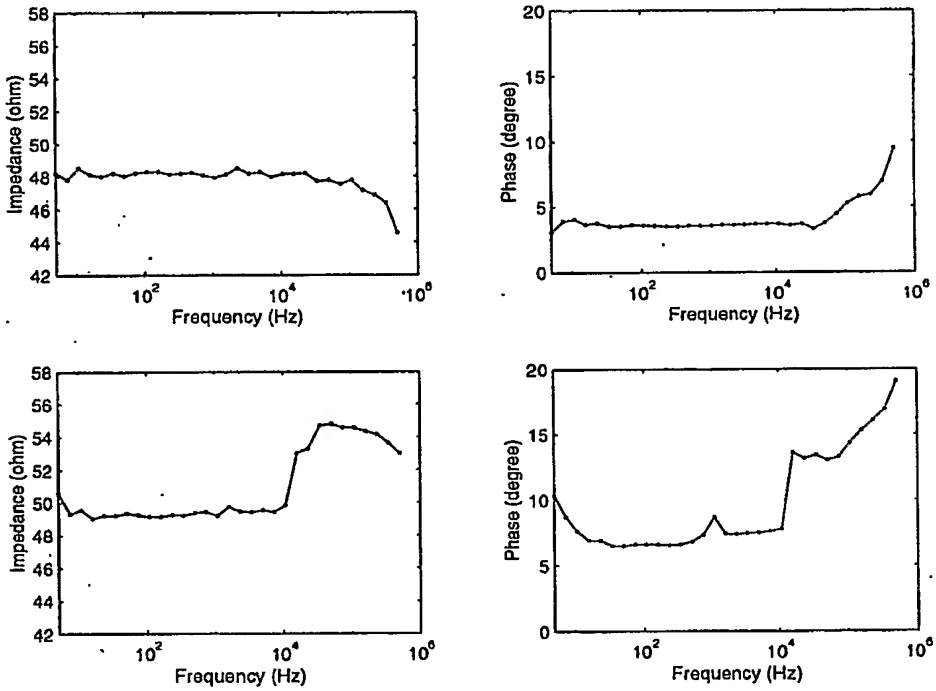

Figure 5.23: Results of the interference testing. The upper figures shows the impedance of the test circuit, with the screen turned off and the lower figures with the screen turned on. Note the characteristic shape of the curve as compared to the raw data plot in 5.21.

As there was no apparent way of ridding the data from noise, no clear conclusions could be drawn at the moment. Instead of doing further data analysis, a search for possible noise sources started. An extremely simple circuit was constructed

using a couple of resistors, with resistance values about the same as the body. The measurements were then repeated exactly as before using this circuit instead of the body. After many measurements conducted at different locations in the experimental room, a possible source was found. One of the computer screens gave the data almost exactly the same interference pattern as observed in the invasive measurement. The effect of the computer screen can be seen in figure 5.23. Since the data processing methods that were used could not rid the data from noise, a possible way to improve the data would be to turn off the screen and then redo the experiment.

5.5.4 Conclusion

Since the results from this experiment showed that the data was too distorted by noise, it was hard to establish a clear correlation between impedance and glucose value. Therefore the question still remains if this method can be used to predict glucose values in vivo, but that cannot be answered without further experiments. The conclusion is that this impedance spectrometer (IBSA) is not suited for an environment with much electrical equipment and the data was discarded.

5.5.5 Further experiments

In order to draw any conclusions at all from this kind of experiment, the noise has to be reduced. One possibility could be to carry out the measurements in a shielded environment or by using another impedance spectrometer that is more insensitive to interference.

5.6 Invasive measurement II

5.6.1 Introduction

Since no conclusion about the correlation between blood glucose level and impedance could be drawn from the previous experiment, the question remained unanswered: Can a correlation be found between intra-muscular impedance and blood glucose level?

The experiment was repeated in almost the same fashion, but with consideration taken to previously earned experiences about interference.

5.6.2 Material and Methods

Setup

The experiment was a repetition of the first one, so only minor changes in the setup was done. To assure that the measurements would not be affected by noise to the same extent, they were performed in a shielded environment (fig 5.24). One of the neighbouring institutions, the Centre for Oral Biology (COB), has a room especially built for measurements that are sensitive to background noise. The room is constructed as a Faradays Cage where the walls, floor and ceiling are covered by metal plates made of μ-metal.

Figure 5.24: Photograph of the measurement setup inside the shielded room.

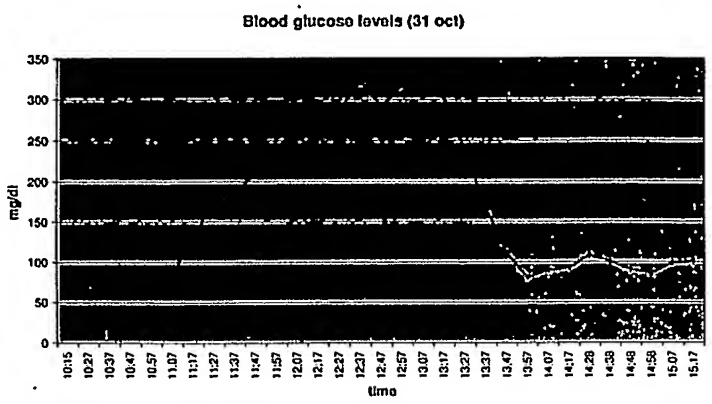

Figure 5.25: Glucose levels during the measurements.

Secondly by replacing the stationary computer with a laptop, the noise from the computer screen was eliminated. Noise was also reduced in other ways, for instance by placing other possible noise sources outside the Faradays Cage.

Experimental procedure

As soon as the needles were put into place, the measurements started. The procedure was the same as before where five registrations were made for each impedance measurement, with one addition. Between each invasive measurement, minimally invasive measurements were performed. The details of these measurements, as well as the data gathered are described in chapter 5.4.

During the measurements, both lunch and 75 g water-free glucose powder was consumed in order to achieve a wide span of blood glucose values, just as in the last experiment (see fig 5.25).

Numerical

Since the raw impedance data contained less noise than before, the mean values of the replicas were considered directly. A PCA model was created based on the mean values of the magnitudes and possible outliers were identified. Thereafter a PLS model was constructed to see if a correlation exists between impedance and blood glucose. Every even sample was used as training data for the model and every other as prediction set. Cross validation of the model was also done using SIMCA.

To validate the model further, the reference glucose vector was permutated a number of times and for each permutation new Q^2_{cum}s and R^2Y_{cum}s were calculated using the same amount of PLS components to validate the statistical significance of the model.

5.6.3 Results

In the last experiment measurements where not conducted during the first hour, since earlier studies [15] suggested that the inflammatory response from the insertion of the needles would affect the measurements significantly. In this experiment however, measurements started right after the insertion. When examining the data, it is clear that the first five measurements (i.e. the measurements carried out during the first 50 minutes) are distorted and thus they were discarded. The conclusions about inflammatory response seem to be applicable in these experiments as well.

The first five frequencies (i.e. 5-23 Hz) were excluded, since these were disturbed. It is known that low-frequency measurements are hard to get noise-free. This was observed in the last experiment. As observed in the loading plot (fig 5.26), the two highest frequencies (340 and 500kHz) are separated from the rest, indicating that these frequencies were affected by noise as well and hence they were excluded.

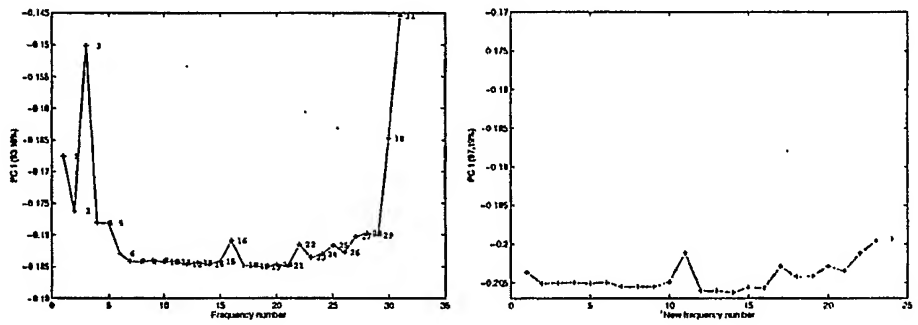

Figure 5.26: Loading plots before and after excluding the problematic frequencies. The frequencies range from 5 Hz to 500 kHz in the left figure and 34 Hz to 232 kHz in the right.

After examining the data with PCA and not finding any apparent outliers the data was fitted to the glucose values using PLS. Looking at the R^2Y_{cum} and Q^2_{cum} values after cross validation, the conclusion was drawn that three components should be used. As seen in fig 5.27, increasing from three to four components will capture a higher variance in Y, but without increasing the predictability. Q^2 for the fourth component is highly negative, meaning that if this component was included in the model, it could become over-fitted.

Figure 5.28 shows the result of the 200 permutations of the Y data. For each permutation new values for R^2Y_{cum} and Q^2_{cum} have been calculated. The fact that all the new R^2Y_{cum} and Q^2_{cum} values remain beneath the values calculated for the model indicates its statistical significance.

Another validation was performed using every second measurement for constructing the model and the rest for prediction validation. The results of this are presented in a BD error grid in fig 5.29, to estimate the clinical significance of the

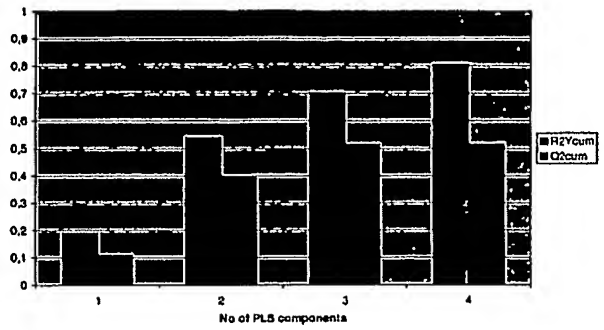

Figure 5.27: R^2Y_{cum} and Q^2_{cum} for increasing number of PLS components.

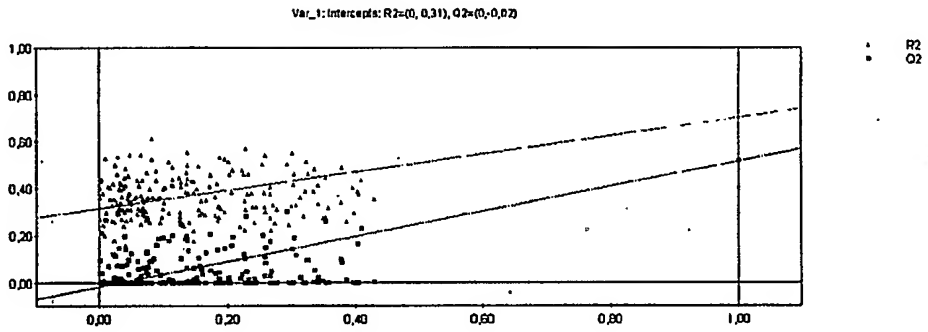

Figure 5.28: The results of the permutation validation. The y-axis represents R^2Y_{cum} and Q^2_{cum} and the x-axis shows the correlation between the permuted and original glucose values (Y data). The original R^2Y_{cum} and Q^2_{cum} are the rightmost values. $\triangle = R^2Y_{cum}$ and $\square = Q^2_{cum}$

model.

5.6.4 Discussion

Conducting the experiment in a shielded environment had a substantial effect on the data. The interference was clearly reduced and no apparent outliers could be found. Some interference still remained, but it did not affect the measurements as much as in the previous experiment. The remaining noise was most likely due to the fact that some noise sources were still present within the shielded environment. The instruments used needed two personal computers and although laptops were used to avoid the interference from ordinary computer screens, the computers still were possible noise sources. If the measurements are to be even less noisy, other

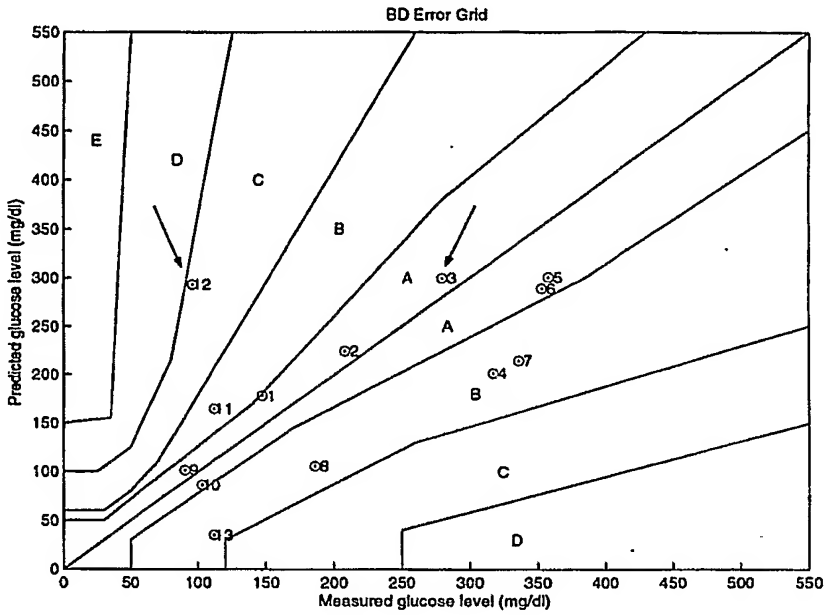

Figure 5.29: The resulting prediction presented in a BD error grid to show clinical significance. The arrows points out two problematic points explained in Discussion.

equipment than the IBSA has to be used, e.g. a better shielded instrument like the Scibase II.

The correlation between blood glucose level and impedance found in this experiment is quite clear. The Q^2_{cum} is 0.516 indicating that the model must be considered to be significant from a statistical point-of-view. The validation through permutation of the Y data enforced the significance of the model.

Even from a clinical point-of-view, the model based on every other sample seems to be significant. 7 values are categorized as A, 5 as B and only one as C, indicating that this method could be clinically interesting (5.29). However, if it is going to be clinically acceptable and thus interesting to construct a device based on this method, no values should fall outside the A category [26].

The one point falling into category C was investigated further, by looking at the values for T^2 and $Q_{residual}$ for all 13 predicted points (fig 5.30). Although point number 12 remains inside the 95% confidence interval for both T^2 and $Q_{residual}$, it is on the verge of falling outside both. Point 3 on the other hand is outside the 95% confidence interval for $Q_{residual}$, but lies well inside the interval for T^2. They are both clearly separated from the rest.

With all 13 points included, r^2 is calculated to 0.4. Removing point 12 gives an r^2 of 0.68 and excluding point 3 as well 0.7. This might seem strange as point 3 is categorized well inside A, but this clearly show the risk of just using the error grid as an evaluation of the model [27].

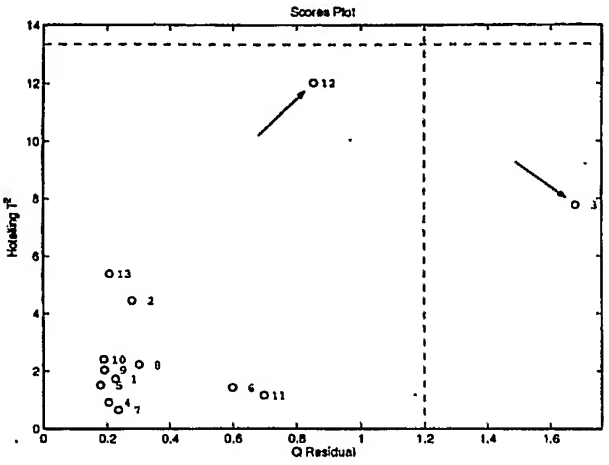

Figure 5.30: Score plot showing the predictions in reference to T^2 and $Q_{residual}$. The dotted lines marks the 95% confidence interval for each variable. The arrows are pointing out possible deviating measurements.

Removing these two points improves the predictability of the model, but since the total number of values in the prediction set amounts only to 13, each excluded point accounts for $\approx 8\%$ of the information. It is also a potential risk in such an approach, as this would improve the appearance of the model, but not its significance. Preferably more data should be collected for more than just one person, as well as during a longer period of time. This would make the statistical analysis more reliable.

5.6.5 Conclusion

None of the previous experiments have given any good predictability for blood glucose concentration. In this experiment, predictions can be made for the first time, indicating that a correlation exists.

5.6.6 Further experiments

Now that a correlation has been established, it would be of great interest to examine the correlation in more detail. There are plenty of questions to be answered:

- Does it exist in other test subjects?
- In what kind of tissue can it be observed and where is it most noticeable?
- Can it be seen during a longer time period?
- What biological factors affect it and how can they be controlled?

To answer some of these questions, a subcutaneous measurement will be conducted, but to tackle the rest, a larger clinical study needs to be conducted.

5.7 Invasive measurement III

5.7.1 Introduction

Now that a correlation has been established, it is time to start investigating where it originates and see if the model can be improved. The main goal of this experiment is to examine whether the correlation between blood glucose level and impedance exists in other kinds of tissue than muscle and skin (chapter 5.6 and [34]).

5.7.2 Materials and Methods

Setup

The by now almost standardized setup was used again with a few changes. Instead of IBSA, the SciBase II was used, since it is much less sensitive to background noise. This made it possible to perform the measurements in an ordinary room instead of the shielded room used in the last experiment. However, since noise is always present in measurements, attempts were made to reduce possible noise sources (e.g. the computer screen that caused problem in experiment 5.5 was turned off).

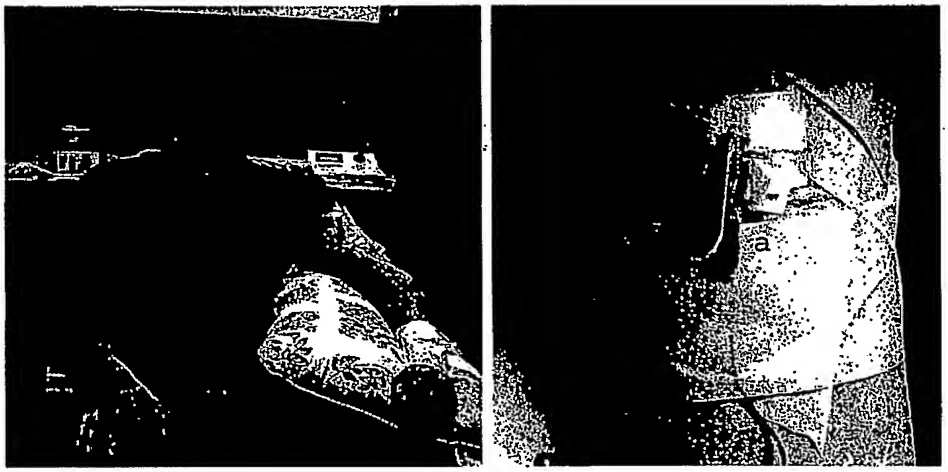

Figure 5.31: Photograph of the measurement setup. In the right figure, a part of the subject's arm has been enlarged: a) connection to the instrumentation, b) entering point for the needle and c) exiting point for the needle.

Since the SciBase II uses two-point impedance measurements, only two electrodes were needed. The two needles were placed subcutaneously in the upper and lower part of the left arm of the same volunteer as in previous experiments. To assure that the needles would not cause more than an initial inflammatory response, the sharp point of the needles were pushed through, so that they exited the skin a few centimeters from the insertion site (see fig 5.31). Blood samples were taken

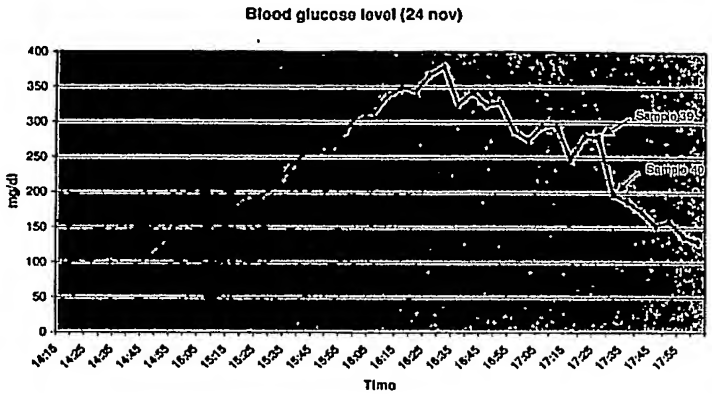

Figure 5.32: Blood glucose levels during the measurements. Sample 39 and 40 are marked, and discussed later, because of the extreme fall of glucose level between them (approximately 80 mg/dl drop in five minutes).

from the finger tips of the right hand and were used for measurements of blood glucose concentration.

Experimental procedure

The measurements did not start until two hours after the incision of the needles. Both glucose level and impedance values were registered simultaneously every five minutes during the entire experiment, that lasted almost four hours. For each impedance measurement, two registrations were done to compare the deviation within each measurement with the deviation between consecutive ones.

Lunch was consumed in the beginning of the measurements, and half way through 75 g of water-free glucose powder dissolved in a glass of water was consumed to achieve a wide span of blood glucose concentrations in the subject (see fig 5.32). Insulin was administered when the blood glucose level reached approximately 300 mg/dl.

At the end of the experiment, the subject was observed during a longer time period, to assure that the subject returned to and remained in euglycaemia.

Numerical

The two replicas from the impedance measurements were examined both in a raw data plot and in a PCA score plot. Possible outliers were also identified in the score plot. After this, a PLS model was constructed and R^2Y and Q^2 were calculated using the same cross validation method as before. Permutations of the Y data

were also performed to validate the model further. In the end a PLS model was constructed from every other sample and the rest were used as a prediction set.

5.7.3 Results

No measurements had to be discarded because of the initial inflammatory response in this experiment, since the measurements did not start until two hours after the insertion of the needles. A PCA score plot was constructed anyhow to reveal possible outliers later during the experiment. The seven last measurements (sample 40-46) were found to be outliers (see fig 5.33) and were discarded. No frequencies were found to deviate substantially from the rest and thus all frequencies were included in the model.

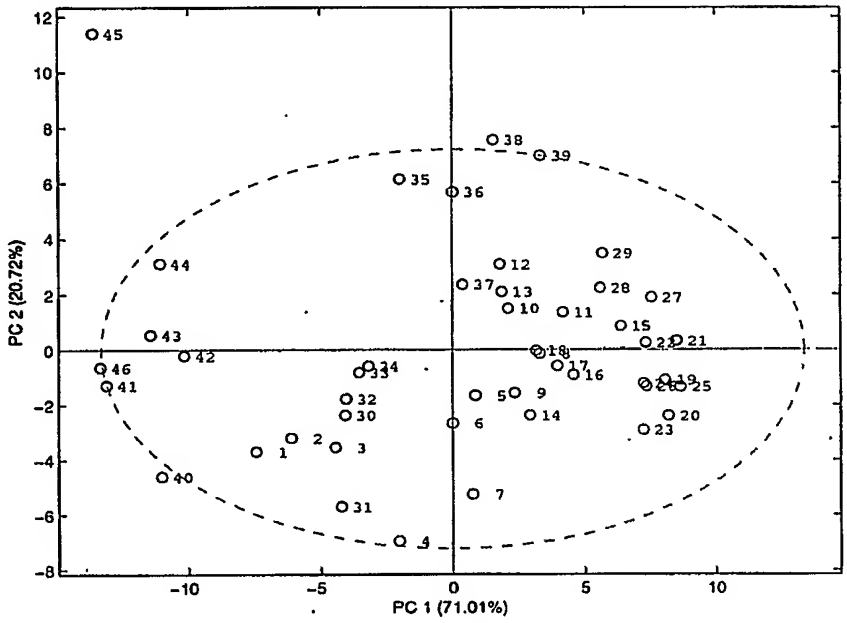

Figure 5.33: PCA score plot of the two first principal components, where the points 1-46 represent the measurements. Notice that points 40-46 are clearly separated from the rest.

Thereafter a PLS model was created on the first 39 measurements. Cross validation was performed and gave the values for R^2Y_{cum} and Q^2_{cum} shown in figure 5.34(left) as well as RMSECV shown in figure 5.34(right). From this figure, the conclusion was made that four components should be used, since increasing from four to five PLS components only gave a minor change in Y variance and Q^2.

For further validation, the Y data was permuted 200 times using the same randomizing seed as before. New values for R^2Y_{cum} and Q^2_{cum} were calculated for each permutation. As seen in fig 5.35, all the new values remained well below

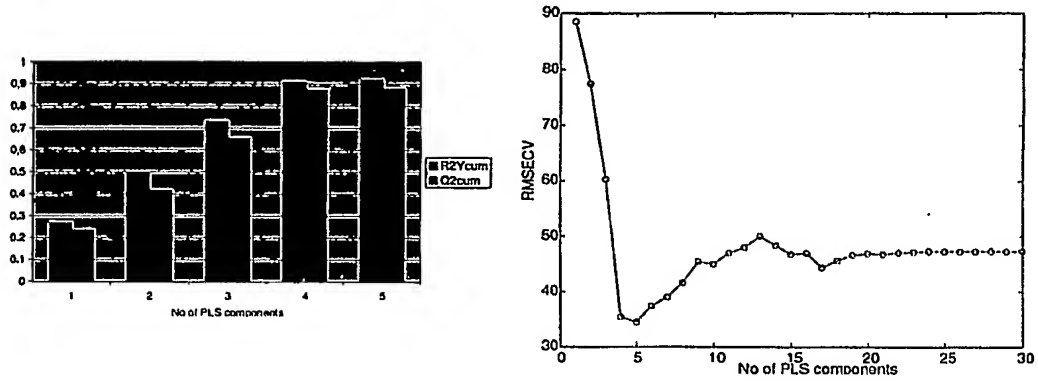

Figure 5.34: R^2Y_{cum} and Q^2_{cum} (left) and RMSECV (right) for increasing number of PLS components.

the original R^2Y_{cum} and Q^2_{cum}, which is another indication of an accurate model:

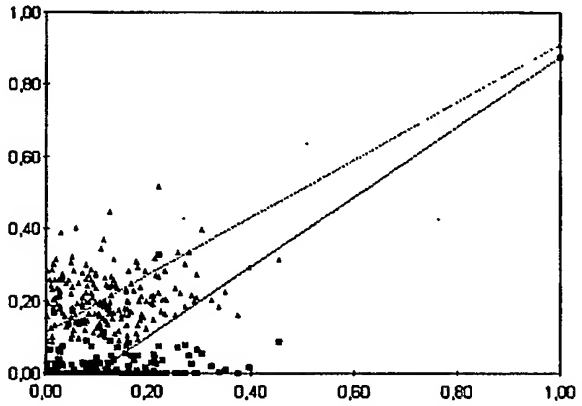

Figure 5.35: The results of the permutation validation. The y-axis represents R^2Y_{cum} and Q^2_{cum} and the x-axis shows the correlation between the permuted and original glucose values (Y data). The original R^2Y_{cum} and Q^2_{cum} are the rightmost values. $\triangle = R^2Y_{cum}$ and $\square = Q^2_{cum}$

Another validation was performed as before, where half the data were used as a training set and the other half as a prediction set. The result from this prediction is shown in a BD error grid in figure 5.36.

At last, an attempt was made at predicting future blood glucose values, where the 30 first measurements (i.e. first 150 minutes) were used to predict the nine last (i.e. last 45 minutes), see fig 5.37. 30 points were chosen for the training set in

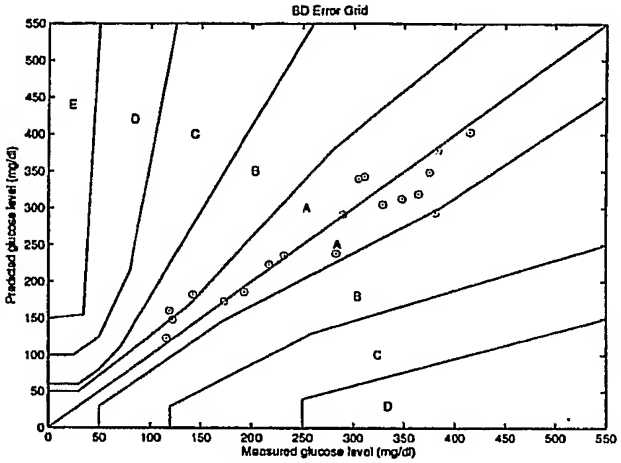

Figure 5.36: The resulting prediction based on every other sample, presented in a BD error grid to show clinical significance.

order to assure that the training set would span the whole range of glucose levels. That left 9 points for the prediction set. When looking at the error grid, the future prediction looked clinically significant, but the statistical analysis gave a very poor value for r^2 of 0.007.

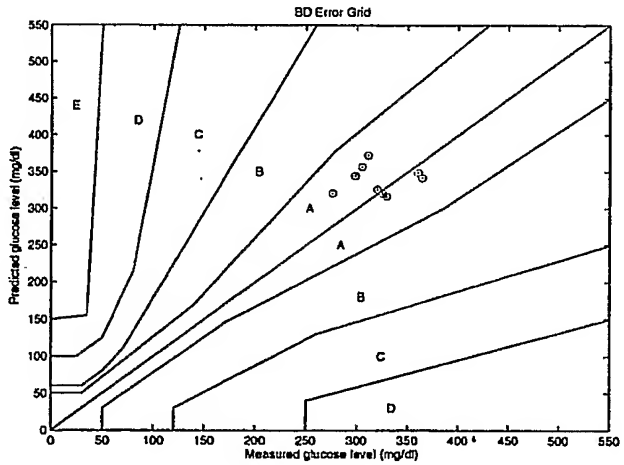

Figure 5.37: Future prediction of glucose concentration based on historical values, presented in a BD error grid to show clinical significance.

5.7.4 Discussion

Upgrading the equipment used from IBSA to Scibase II had a remarkable effect on the impedance data. This experiment was not conducted in a shielded environment, but still the data contained almost no interference. This is shown by the fact that both replicas for each measurement were almost identical, which could be observed both in the raw data itself and in a PCA score plot with both replicas included. On one occasion, the replicas were clearly separated in the score plot. Since the replicas were registered only five seconds apart, they should be close to identical. Observing this kind of separation is a good way to find erroneous measurements and hence this measurement most likely should be discarded.

As can be observed in figure 5.33, all points up to number 39 lie close to both their predecessor and successor. However, between 39 and 40 a substantial leap has occurred. This might be due to a movement of the needles, since measurements 40 to 46 were made after the subject had made a visit to the toilet. A movement of the needle will cause a new inflammatory response that will interfere with the measurements.

The circulation in the arm of the subject could possibly be reduced during the measurement, since the subject is almost immobilized. Directly after the toilet visit, the subject stimulated the circulation in the right arm (i.e. the arm used for blood sampling, not the one with the needles) by flexing it several times. The increased circulation in the hand of the subject could explain the extreme fall of blood glucose concentration seen between sample 39 and 40 in fig 5.32. A reduced circulation, as before the flexing of the arm, could lead to an inaccurate invasive blood glucose measurement, but the question is how crucial this error would be.

The Q^2_{cum} calculated for the model by using cross validation was 0.875 and R^2Y_{cum} 0.909 for four PLS components. This is a close to excellent model when only considering these values and thus the correlation between blood glucose concentration and impedance is even stronger than in previous experiments.

Using every second point for building the model and the rest for prediction gave a very significant model from both a clinical and statistical point-of-view. In the BD error grid (fig 5.36) only 3 points were categorized outside A and all these were categorized in B. The correlation coefficient r^2 had a value of 0.9. However this kind of model building will give an *overly positive* picture of reality, since consecutive points are closely linked to each other, which also can be seen in the PCA score plot (fig 5.33).

Interesting for real applications is not whether every second point can be predicted or not, but if future predictions of glucose concentration can be made. Therefore one must attempt to predict for instance the last measurements, using the previous points for training. When looking at figure 5.37, it almost seems to be working from a clinical point-of-view, but the correlation coefficient r^2 remains unsatisfactory low. This could be explained in three separate ways:

- The model does not contain enough values to test and describe whether it works for future prediction or not. In order to evaluate if the model is working for this kind of prediction, a larger data set is needed for the prediction, a data set that spans the whole variance of glucose concentration. The data set that were used for prediction in this attempt contained only nine points, with glucose levels between 250 and 325 mg/dl, whilst the training data has glucose levels between 100 and 350 mg/dl.
- Different biological phenomena might distort the measurements in such a way that future predictions cannot be made without taking them into consideration. To understand these possible phenomena, more data is needed. Not only more impedance data, but also data of other biological parameters than glucose concentration.
- Another possibility is that future prediction does not work at all.

It is also important to remember that this model is based on only one subject. To be able to draw any general conclusions about future predictions, data from a population is needed to be sure that this is not merely a single event.

5.7.5 Conclusion

The correlation between blood glucose concentration and impedance has been established once again. This time in a subcutaneous measurement, showing that the correlation can be found in other tissues than muscle or skin. The correlation is even clearer than in any of the previous experiments, suggesting that one should measure beneath the skin.

The only kind of predictions to facilitate a foundation for a new SMBG device is future predictions and they are still *not* possible to make. To solve this problem, more data is needed and not only from one subject.

5.7.6 Further experiments

Since this experiment also was performed on only one subject, several of the questions mentioned in the previous chapter still remains. To answer those, a clinical study was suggested and since the conclusion of this experiment is that more data is needed, it is now even more essential to perform such a study.

Chapter 6

Final Results

The search for a correlation between impedance and glucose concentration started in a laboratory environment. The impedance of a physiological saline/glucose mixture was measured for different glucose concentrations and a clear correlation was found (chapter 5.1).

To investigate the correlation further and find out if it holds in a more body-like environment, the experiment was repeated with a cell structure. Oasis blocks were soaked with the saline/glucose solution and the impedance was registered for different glucose concentrations. Once again a correlation was established (chapter 5.2).

Now that a correlation had been established in the laboratory environment, the next step was to measure non-invasively on the body. An attempt was made at linking skin impedance with blood glucose concentration, since this link has been established in earlier studies [33, 34]. A clear drift in the measurements between days made the correlation hard to find (chapter 5.3).

Since the drift was most likely due to changes in the top layers of the skin (see page 50) a new kind of probe was introduced. This made it possible to reduce the dominant dielectric effect of the horny layer and thus minimize the effect of skin changes on the measurements. However, practical problems with the probe made it hard to distinguish any clear correlation (chapter 5.4).

The first two experiments established a correlation between impedance and glucose concentration generally, but the attempts made at establishing the correlation between *skin* impedance and *blood* glucose concentration were not successful. Therefore measuring impedance underneath the skin seemed to be a logical approach and so the first invasive experiment was conducted. Interference from other electrical equipment distorted the measurement data severely and made the correlation unattainable (chapter 5.5).

To reduce the interference, the invasive experiment was repeated in a shielded environment at a neighbouring institution, the Centre for Oral Biology. This time a correlation was established between the impedance in a muscle and blood glucose concentration (chapter 5.6).

In both the first and the second invasive measurement an older instrument

called IBSA was used. In order to lessen the need for a shielded environment and improving the quality of the data, another experiment was performed using a new instrument, that is virtually immune to external noise, called SciBase II (the instrument that was used in both the skin impedance experiments). The electrodes were placed in subcutaneous fat in order to examine if the correlation could be found in other tissue than muscle or skin. An even clearer correlation was found in this experiment (chapter 5.7).

For clinical applications the ability to predict future glucose levels is essential. An attempt to predict future glucose levels was made, which was clinically, but not statistically, significant. The reason for the shortcoming has not yet been determined (see chapter 5.7.4 Discussion, page 75).

Chapter 7

Final Discussion

The experiments conducted in this thesis strongly suggest that a correlation can be found between impedance and blood glucose concentration. Since not enough data has been collected, the origin of the correlation has not been fully investigated.

Through laboratory experiments (chapter 5.1 & 5.2) it has been shown that glucose concentration modulates the impedance level in a solution made of physiological saline and glucose. *In vivo* however, other factors will be of importance as well. The blood glucose level is crucial for a number of processes in the human body. Both hypo- and hyperglycaemia will provoke several bodily responses, which might in turn alter the impedance of different kinds of tissues. For instance, one of the effects of hypoglycaemia is the release of epinephrine, which will cause the blood circulation to increase. In type 1 diabetics the bodily response of epinephrine release will diminish as the time after diagnosis goes by, making it an important factor to be included in further studies. Long gone hyperglycaemia is also of importance, since it will cause ketoacidosis with lowered pH-value as *one* result [3]. Numerous other factors are likely to have an effect on the measured impedance values and thus they must be investigated as well.

Among the experiments performed the correlation was most clearly seen with the electrodes placed subcutaneously (chapter 5.7), but could also be seen with intra-muscular electrodes (chapter 5.6). The reason for the weaker correlation could be equipment related, since an older instrument was used for the intra-muscular measurements, whilst the subcutaneous measurements were performed with a new and improved instrument, the SciBase II [28].

The correlation has also been detected in skin in earlier studies [34]. The drift between days, emanating from several factors that are described in more detail in 5.3.4, made such a correlation unattainable in these experiments. Going beneath the horny layer with a spike probe did not give any interesting results, because of wear out symptoms of the probe (chapter 5.4). The possibility of finding a correlation in skin measurements has not been put aside completely, since not enough data was collected to draw any real conclusions.

Throughout these experiments, the correlation seems to be strongest inside the

body. This needs to be further investigated, since some kind of correlation has been found in all tissues measured on. One possibility is that the correlation has its origin in specific tissue inside the body, but that it spreads out and can be observed in various degree in all kinds of tissues.

If the modulation of impedance level is mainly caused by altered ionic mobility, as a consequence of altered turbidity of body liquids, the change would start in principle everywhere with a delay of minutes due to diffusion time for glucose molecules. Other factors might then be considered as artefacts, which, if known, might be possible to compensate for by additional calibration.

In the end of the last experiment an attempt was made at predicting future glucose levels, which is the only kind of prediction that is really clinically interesting for SMBG applications. The results look promising from a clinical point-of-view, but once again the lack of data makes the statistical evaluation inconclusive (discussion 5.7.4).

Chapter 8

Final Conclusion

These experiments strongly suggest that a correlation exists between blood glucose level and electrical impedance in human tissues. In order to draw conclusions about the general applicability of the correlation, a clinical study needs to be performed, since these experiments were only conducted on one test subject.

The fact that glucose seems to modulate the impedance level in vivo is also interesting for other applications where impedance is used for modelling different body compartments, such as classical body composition analysis, as well as electrical impedance tomography (EIT).

Chapter 9

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Appendix A

Glossary

Arteriosclerosis	A group of diseases characterized by thickening and loss of elasticity of arterial walls.
Bioimpedance	The electrical impedance of tissue.
Dermis	The layer of the skin deep to the epidermis, consisting of a dense bed of vascular connective tissue.
Diabetes Mellitus	A chronic syndrom of impaired carbohydrate, protein and fat metabolism owing to insufficient secretion of insulin or to target tissue insulin resistence.
Dielectric	A non-conducting substance or material through which electrostatic lines of force may pass.
ECF	Extra Cellular Fluid, fluid outside the cells.
Electrical Impedance	An electrical property describing the relationship between voltage and current in a given material.
Epidermis	The outermost and nonvascular layer of the skin.
Euglycaemia	Blood glucose levels within the normal range.
FASS	"Farmaceutiska Specialiteter i Sverige", Pharmaceutical Specialities in Sweden. A medical dictionary of all available pharmaceuticals in Sweden.
HbA1c	A glycosylated hemaglobin A, having a hexose attached to the N-terminal of its β-chain; its levels are increased in poorly controlled diabetics.
Horny layer	The outermost layer of the epidermis, consisting of cells that are dead and desquamating.

Hyperglycaemia	An abnormally increased content of glucose in the blood.
Hypoglycaemia	An abnormally dimished concentration of glucose in the blood.
ICF	**I**ntra **C**ellular **F**luid, fluid situated inside a cell.
In vitro	In an artificial enviroment.
In vivo	Within the living body.
Insulin	A protein hormone secreted by the beta cells of the pancreatic islets that serves as a hormone signal of the fed state; it is secreted in response to elevated blood glucose and amino acids.
Pancreas	A large and elongated gland situated transversely behind the stomach, between the spleen and the duodenum.
Pathophysiology	The physiology of disordered function.
PCA	**P**rincipal **C**omponent **A**nalysis, see chapter 4.5
Pharmaceutical	1. Pertaining to pharmacy or to drugs. 2. A medicinal drug.
Physiological	Pertaining to physiology
Physiology	The science which treats of the the functions of the living organism and its parts, and of the physical and chemical factors and processes involved.
PLS	**P**artial **L**east **S**quare regression, see chapter 4.6
Retinopathy	Disordered function of the retina.
Saline	Solution of water and NaCl, physiological saline solution has a concentration of 0.9% NaCl.
SMBG	**S**elf **M**onitoring of **B**lood **G**lucose
Subcutane	Beneath the skin

Most of these explanations are cited from either *Encyclopedic Dictionary of Electronics and Nuclear engineering* [12] or *Dorland's Illustrated Medical Dictionary* [13].

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APPENDIX B

The technique referred to as the "four point technique" as described in Chapter 3 beginning at page 22, has been used in body composition research and analysis including examinations of lean body mass, fat mass, extracellular water, intracellular water, etc., as well as alterations of skin and oral mucosa and cardiac function: see Min et al., (2003) *International Journal of Bioelectromagnetism*, Vol. 5, No. 1, pp.53 –56. In that publication, it was shown that a 4-pointed "fork" may assess the viability of organs such as kidneys, hearts, etc. before transplantation. Invasive 2-point analysis, also known in the relevant art, would also work above around 10 kHz, but certainly not below 1 kHz, unless very special surface preparation of the electrodes is used due to the frequency dependent electrode/tissue interface, which could be to some degree compensated by larger electrodes, if possible in the allotted space of investigation, or by increasing the surface area of electrodes by depositing a fractal or microgranuled surface layer.

In the 4 point analysis, for example, two electrodes, such as those provided in Figure 3.7 that are shown as "rings" and which can be located outwardly from the remaining two electrodes, are used to inject electric current while the remaining inwardly located electrodes, are used to detect the resulting voltage created by the current travelling through the resistive or impedic tissue, according to Ohm's law. In body composition, there can be a choice between whole body analysis, such as, for example, wrist to ankle diagonally through the body with two electrodes on each wherein one electrode provides current injection and one electrode for voltage sensing, or body segment, such as, for example, a part or portion of one arm or one leg, having 4 electrodes or "rings" at or along the body segment. There are less artifacts, such as noise, mainly from body movement, internal or external, and unstable electrode/tissue contacts, etc., with measurement involving the body segment, since the trunk, in particular the thorax section with, for example, its moving heart and lungs, as well as the peristaltic movement of intestines in the bowel that may generate artifacts, are eliminated. Still, in whole body most of the contribution comes from arm and leg because the impedance in the extremities is much higher than in the trunk.

With respect to the invasive measurement described herein, which is intended to eliminate the skin in the measurement process, it is now possible to obtain the same information

using the four point technique as known in the relevant art and described above since by 4 point technique the poor contact problem originating from the skin is eliminated. Now, enough current can be injected with a constant current generator. However, in the immediate vicinity of the electrode(s), such as, for example, within millimeters or centimeters, and depending on size and geometry of electrodes as well as their distances from the current electrodes, namely those electrodes in the four point technique that generate or produce the current, a number of artifacts from the electrode/tissue interface may be added to the desired tissue properties, so that if the voltage is sensed with the same electrodes, these artifacts will be added to the signal, making it more noisy, as would be understood in the art. All sections/materials/tissues in the electric current trajectory are in series connection thus adding to the sensed signal, while if sensing is only over the desired section, the other sections are excluded from being sensed in the measurement, although they carry electricity like the cables to the 4 point instrument. If the voltage is sensed by separate electrodes some distance away from the "immediate zone" these artifacts are circumvented. Now, since voltage can be sensed with a practically ideal voltage meter, e.g. very high input impedance so that voltage is sensed without stealing energy from the source, no current will be floating through the voltage electrodes, namely the electrodes that measure the voltage (and their electrode/tissue interface), and thus there will be no voltage drop across that interface - including the skin. If enough distance between current injecting or current electrodes, the injected test current will be spread through the whole body segment, and the voltage electrodes will sense a potential representative of a cross section through the whole body segment, so that the data will be equivalent to inserting invasive electrodes along the equipotential sensing lines ending at the voltage electrodes. In the master thesis, we used 2 inserted needle electrodes made by hypodermic cannula, and 2 outer injection electrodes in an early experiment with a noisy 4-point impedance spectrometer or noisy IBSA, but in the final experiments a 2 point measurement was used with the noise reducing 2 point device, the SciBase II. It will be understood that with a good 4-point machine, the same data would have been obtained with only non-invasive 4 electrode system, like e.g. 4 rings or 4 ECG-type electrodes along a body segment like an arm or a leg. The difference between 2 and 4 point becomes increasingly important when going down or decreasing in frequency and measuring only non-invasive, such as, for example, when the contribution from the skin barrier rises, as disclosed in Nicander I. *Electrical Impedance Related to Experimentally Induced Changes of Human Skin*

and Oral Mucosa, (1998) Ph.D. Thesis, Karolinska Institutet. In the MHz frequency region, the stratum corneum containing the functional skin barrier would appear as the insulating layer of a capacitor, and the impedance of a capacitor is inversely proportional to frequency. Of course, leaving 4 electrodes just under the skin would work equally well - implantation. Four implanted electrodes are better than two, because electrode/tissue interface artifacts will be eliminated just the same, and in the implantation case such artifacts might be caused by degradation of electrode, encapsulation of alien material, tissue reaction (mechanical or chemical or even allergic). Implanted electrodes, which could be permanently or temporary implanted, might change due to material/tissue interaction and 4-point would again eliminate the problem of unstable contacts. Permanently implanted, a day or so attached outer electrodes, could be used for very short application of electrodes, such as, for example, during measurement, to obtain the same data. For example, the longer resident electrodes could be combined with an insulin pump, in which case the 4 electrodes could be part of the pump encapsulation, as is the case for other such devices, such as, for example pacemakers.